THYROID HORMONE IN BRAIN AND BRAIN CELLS

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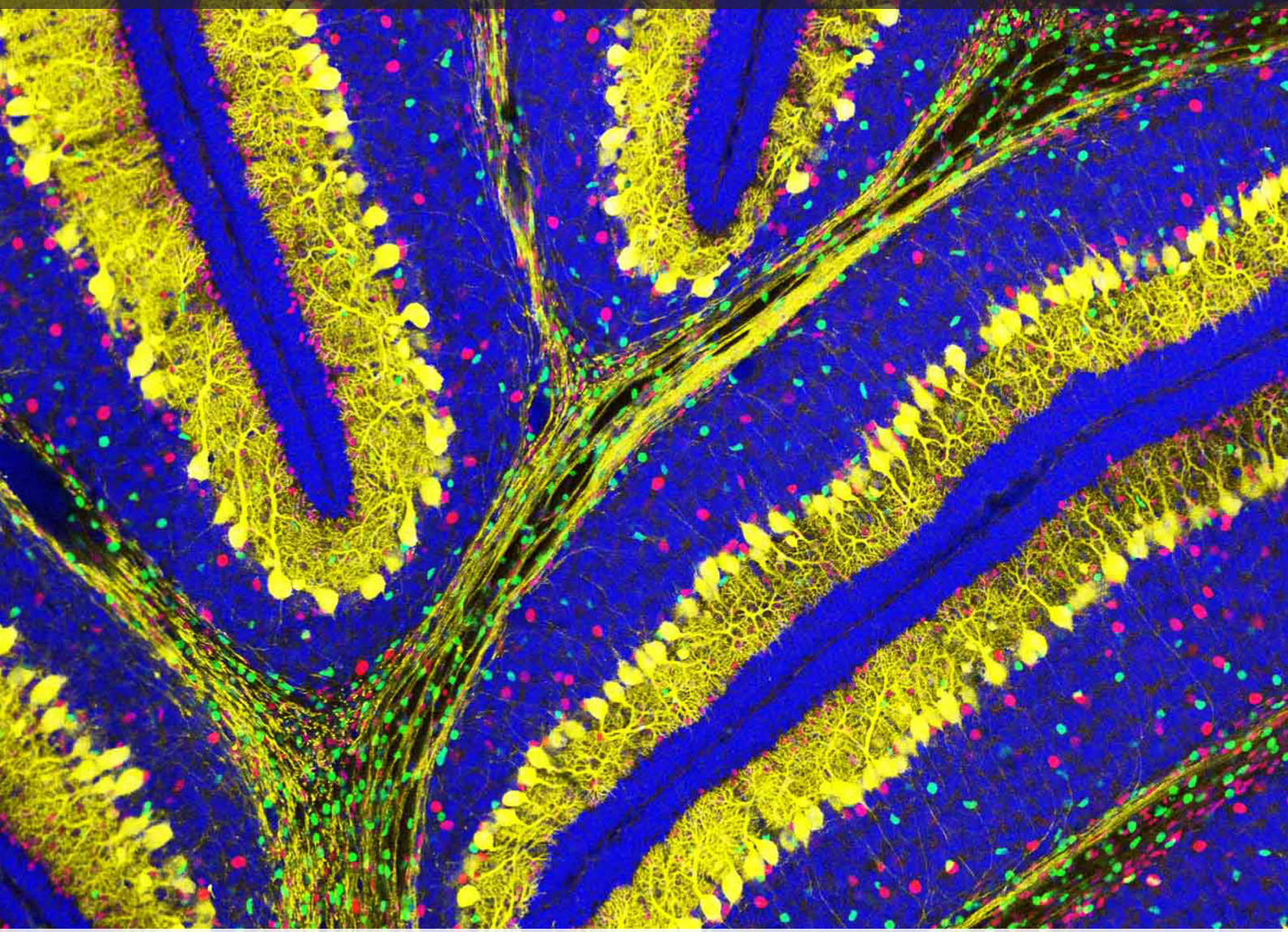

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THYROID HORMONE IN BRAIN AND BRAIN CELLS

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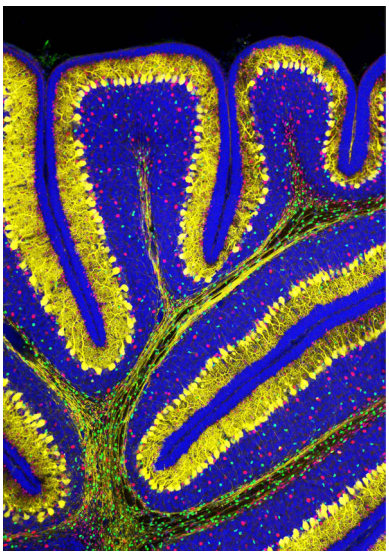

Hypothyroid cerebellum. Sagittal section of mouse cerebellum at post-natal day 15. The external granular layer (DAPI, blue) remains thick. Arborization of Purkinje cells (yellow) is reduced. Oligodendrocytes progenitor cells (Olig2+ green) and GABAergic neuron progenitors (Pax2+) differentiation is retarded. Picture by Teddy Fauquier.

Thyroid hormone signaling has been known for a long time to be required for proper neurodevelopment and the maintenance of cognitive functions in the adult brain. As thyroid hormone excess or deficiency is usually well handled by clinicians, research dedicated to the neural function of thyroid hormone, have not been a priority within the field. This is changing mainly for two reasons. First, new genetic diseases have been discovered, altering thyroid hormone signaling in brain (THRA, MCT8, SBP2), with neurodevelopmental consequences which are currently incurable. Second, there is a growing concern that exposition of the general population to environmental chemicals able to interfere with thyroid hormone signaling compromises children neurodevelopment or induces central disorders in adults. Finally thyroid hormone is acting directly on gene transcription, by binding nuclear receptors, and is therefore an interesting entry point to identify genetic programs controlling brain development and function. Reaching a broad understanding of the multiple processes involving thyroid hormone in brain is a tremendous task which will necessitate a multidisciplinary approach: animal genetics, molecular biology, brain imaging, developmental biology, genomics, etc… This topic will be the occasion to combine recent contributions in the field and to identify priorities for future investigations.

Due to devastating consequences of congenital hypothyroidism, the neurodevelopmental consequences of altered thyroid hormone signaling have been extensively studied over the years. The discovery of new genetic diseases, the concern about the possible neurotoxicity of

environmental thyroid hormone disruptors, recently renewed the interest for an important research field. This Ebook gathers reviews and original data from experts in various disciplines. It provides a broad view of ongoing research and outlines key issues for future investigation.

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EDITORIAL
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Editorial: "Thyroid hormone in brain and brain cells"

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Keywords: thyroid hormones, thyroid hormone signaling, gene transcription, editorial, neurodevelopmental function of TH

Thyroid hormones (TH) function in brain has been known for a very long time. In 1813, Jean-François Coindet, a Swiss physician, made the discovery that iodine was efficient at treating goiter and cretinism, a disease associated to mental retardation, which was endemic in his country. This started a persisting tradition of research, which first identified TH [first thyroxine (T4) and then 3,5,3′-tri-iodo-L-thyronine) (T3)] as the active iodinated compounds, which early deficiency explained cretinism. It also revealed a number of other functions for TH, not only during development but also in adult brain. It is now well established that most brain cell types need TH for a proper and timely differentiation. What makes the situation in brain different than in other organs is that the consequences of TH deficiency become quickly irreversible. After the elucidation of the corresponding signaling pathway, and the identification of the two genes, now called THRA and THRB, which encode the nuclear receptors of T3 (TRs, including TRα1, TRβ1, and TRβ2), one would expect that this research field would eventually run out of unsolved mysteries. This is far from being the case and it seems that new questions keep arising all the time. This special issue is a snapshot on some of current hot topics, which bring a stimulating overview of the current situation.

One key aspect of the neurodevelopmental function of TH, bringing major complications, is that TH does not freely circulate in all brain areas and cell types, as originally postulated. TH signaling seems therefore to be heterogeneous and dynamic in brain. First of all, local metabolism by deiodinases can modulate the availability of TH. This has been exemplified in anterior cortex (1), and studied in details in inner ear (2). Second, specific transporters play a major role for the distribution of TH. Therefore, although most brain cells possess at least one of the TR, the levels of TH in serum provide little indication for the TH-signaling level in different brain areas. The physiopathological relevance of the question of TH transport in brain is best illustrated by the Allan–Herndon–Dudley syndrome, a devastating genetic disease because of a genetic mutation in MCT8, a gene encoding one of the TH transporters (3). Schroeder and Privalsky provide a clear introduction to this difficult question, which involves local metabolism of TH by deiodinases, and specific transporters required for TH to cross the blood–brain barrier or to reach the cell nucleus (4). Anticipating recent reports for the importance of non-genomic pathways for TH signaling in brain (5) they raise the hypothesis that T4 itself may be more than a prohormone, having a function different from T3 in some situations. They also explain that differential expression of coregulators may modulate TH signaling during development, a possibility that has not yet been extensively explored (6, 7). Muller and Heuer (8) provide a novel and extensive description of the main TH transporters expression patterns in mice. These new data confirm that the transporter can potentially generate a very heterogeneous distribution of TH in brain. Wirth et al. (9) provide a comprehensive overview of the growing knowledge on TH transporters in brain, in various vertebrate models. They also discuss the possibility that other iodinated compounds, which are also transported in brain, may have a neglected function, independent of the classical TR pathway.

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Although neurons are the primary target of T3 actions, most of the T3 present in brain is made by T4 deiodination, which takes place predominantly, if not exclusively, in glial cells: the tanycytes lining part of the third ventricle surface and in the astrocytes throughout the brain. Morte and Bernal (10) show that how the combination of primary cell cultures, genome-wide expression analysis, and mouse genetics recently revealed a dynamic evolution of this astrocytes–neurons dialog during neurodevelopment. This also led to the puzzling conclusion that the source of T3 matters: some genes, which expression is down-regulated by T3, would respond differently, depending on whether T3 crossed the brain–blood barrier, or was produced by local deiodination of T4. A general picture emerges, where TH become much more than a trophic factor, their tightly regulated distribution providing positional information to the developing neurons.

The neurodevelopmental consequences of altered TH signaling have been studied in great details over the years. Cerebellum proved to be a brain area suitable for in-depth investigation in rodent models. The first advantage, compared to other brain areas, is its relatively simple neuroanatomy, which few main cell types. The other is that its maturation takes place at a late stage of brain development, within the first post-natal weeks in rodent, when the circulating level of TH is normally high. This probably explains why the histological consequences of early TH deficiency are particularly dramatic in this brain area. Faustino and Ortiga-Carvalho review the recent progresses in our understanding in the way TH coordinate cellular interactions during this process, and the limited knowledge that we have on TRα1 and TRβ1 target genes in this promising model (11). In an ambitious reflection, Berbel et al. (12) generalize these concepts to cortex development, carefully discussing the relevance of rodent models to human pathology, and placing animal studies in an evolutionary perspective. In-depth examination of T3 regulated genes reveals hidden connection between TH deficiency and major neurodevelopmental diseases: epilepsy, autism, attention deficit hyperactive disorder, and schizophrenia. This landmark review should have far reaching consequences for later investigations, as it outlines that T3 is an essential timer of brain development, and that any alteration in T3 signaling has long-term consequences on neurological and cognitive functions. Remaud et al. (13) show that TH neurodevelopmental functions in brain do not stop after maturation, but persist throughout life, as the differentiation of adult neural stem cells, present in the hippocampus and the subventricular zone, also depends on TH. This leads to the proposal that the known decline of TH levels upon aging, may partially explain several adverse effects on cognitive functions.

One site in the adult brain where TH has been proved to exert a number of important functions is the hypothalamus. This is especially important because hypothalamus is a brain area, which communicates with peripheral organs and a place where many physiological processes can cross-talk. Some TH functions, which are thought to involve peripheral organs, may actually stem in the hypothalamus. One important example is that the control of energy homeostasis, originally believed to result from direct stimulation of liver, muscles, white, and brown adipose tissue. It is now demonstrated that this important function of TH also involves the hypothalamus, which secretes a number of signaling peptides and set the sympathetic tone (14). Three reviews focus on one hypothalamic function of TH, which is an area of intense investigations: the involvement of TH in the so-called seasonal clock, which allow many animal species to reproduce at a specific season. Using a fish model, Ogawa et al. present new data showing that TH can activate, directly or indirectly, the expression of *Kiss2* and *Gnrh* genes in hypothalamus, which are important upstream effectors of the gonadotrophic axis (15). Shinomiya et al. explain how initial studies of the photoperiodic change in gene expression in hypothalamus, performed in quail by the Nagoya group, led to the discovery of a general mechanism, common to vertebrate, which allow to couple the seasonal change in day length and reproduction (16). Dardente et al. highlight several missing links in this general model, which suggest that important contributions are still ahead (17).

All these contributions provide a timely update of an abundant literature, and suggest exciting avenues for new investigations. These will also be stimulated by new questions raised by the discovery of new genetic diseases altering TH signaling in brain (18) and by the concern that some environmental contaminants acting as TH disruptors might compromise normal brain development (19). Most of all, as TH act primarily on gene expression, studies of TH function in brain will continue to provide an outstanding opportunity to explore the basic genetic mechanisms, which govern neurodevelopment and adult brain functions.

References

1. 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**:5550–8. doi:10.1210/en.2010-0450
2. Ng L, Hernandez A, He W, Ren T, Srinivas M, Ma M, et al. A protective role for type 3 deiodinase, a thyroid hormone-inactivating enzyme, in cochlear development and auditory function. *Endocrinology* (2009) **150**:1952–60. doi:10.1210/en.2008-1419
3. Friesema EC, Ganguly S, Abdalla A, Manning Fox JE, Halestrap AP, Visser TJ. Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. *J Biol Chem* (2003) **278**:40128–35. doi:10.1074/jbc.M300909200
4. Schroeder AC, Privalsky ML. Thyroid hormones, t3 and t4, in the brain. *Front Endocrinol* (2014) **5**:40. doi:10.3389/fendo.2014.00040
5. Martin NP, Fernandez de Velasco EM, Mizuno F, Scappini EL, Gloss B, Erxleben C, et al. A rapid cytoplasmic mechanism for pi3 kinase regulation by the nuclear thyroid hormone receptor, TRbeta, and genetic evidence for its role in the maturation of mouse hippocampal synapses in vivo. *Endocrinology* (2014) **155**:3713–24. doi:10.1210/en.2013-2058
6. Yousefi B, Jingu H, Ohta M, Umezu M, Koibuchi N. Postnatal changes of steroid receptor coactivator-1 immunoreactivity in rat cerebellar cortex. *Thyroid* (2005) **15**:314–9. doi:10.1089/thy.2005.15.314
7. Ramos HE, Weiss RE. Regulation of nuclear coactivator and corepressor expression in mouse cerebellum by thyroid hormone. *Thyroid* (2006) **16**:211–6. doi:10.1089/thy.2006.16.211
8. Muller J, Heuer H. Expression pattern of thyroid hormone transporters in the postnatal mouse brain. *Front Endocrinol* (2014) **5**:92. doi:10.3389/fendo.2014.00092
9. Wirth EK, Schweizer U, Kohrle J. Transport of thyroid hormone in brain. *Front Endocrinol* (2014) **5**:98. doi:10.3389/fendo.2014.00098

10. Morte B, Bernal J. Thyroid hormone action: astrocyte-neuron communication. *Front Endocrinol* (2014) **5**:82. doi:10.3389/fendo.2014.00082
11. Faustino LC, Ortiga-Carvalho TM. Thyroid hormone role on cerebellar development and maintenance: a perspective based on transgenic mouse models. *Front Endocrinol* (2014) **5**:75. doi:10.3389/fendo.2014.00075
12. Berbel P, Navarro D, Roman GC. An evo-devo approach to thyroid hormones in cerebral and cerebellar cortical development: etiological implications for autism. *Front Endocrinol* (2014) **5**:146. doi:10.3389/fendo.2014.00146
13. Remaud S, Gothie JD, Morvan-Dubois G, Demeneix BA. Thyroid hormone signaling and adult neurogenesis in mammals. *Front Endocrinol* (2014) **5**:62. doi:10.3389/fendo.2014.00062
14. Lopez M, Alvarez CV, Nogueiras R, Dieguez C. Energy balance regulation by thyroid hormones at central level. *Trends Mol Med* (2013) **19**(7):418–27. doi:10.1016/j.molmed.2013.04.004
15. Ogawa S, Ng KW, Xue X, Ramadasan PN, Sivalingam M, Li S, et al. Thyroid hormone upregulates hypothalamic kiss2 gene in the male Nile tilapia, *Oreochromis niloticus*. *Front Endocrinol* (2013) **4**:184. doi:10.3389/fendo.2013.00184
16. Shinomiya A, Shimmura T, Nishiwaki-Ohkawa T, Yoshimura T. Regulation of seasonal reproduction by hypothalamic activation of thyroid hormone. *Front Endocrinol* (2014) **5**:12. doi:10.3389/fendo.2014.00012
17. Dardente H, Hazlerigg DG, Ebling FJ. Thyroid hormone and seasonal rhythmicity. *Front Endocrinol* (2014) **5**:19. doi:10.3389/fendo.2014.00019
18. Bochukova E, Schoenmakers N, Agostini M, Schoenmakers E, Rajanayagam O, Keogh JM, et al. A mutation in the thyroid hormone receptor alpha gene. *N Engl J Med* (2012) **366**:243–9. doi:10.1056/NEJMoa1110296
19. Miller MD, Crofton KM, Rice DC, Zoeller RT. Thyroid-disrupting chemicals: interpreting upstream biomarkers of adverse outcomes. *Environ Health Perspect* (2009) **117**:1033–41. doi:10.1289/ehp.0800247

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An evo-devo approach to thyroid hormones in cerebral and cerebellar cortical development: etiological implications for autism

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The morphological alterations of cortical lamination observed in mouse models of developmental hypothyroidism prompted the recognition that these experimental changes resembled the brain lesions of children with autism; this led to recent studies showing that maternal thyroid hormone deficiency increases fourfold the risk of autism spectrum disorders (ASD), offering for the first time the possibility of prevention of some forms of ASD. For ethical reasons, the role of thyroid hormones on brain development is currently studied using animal models, usually mice and rats. Although mammals have in common many basic developmental principles regulating brain development, as well as fundamental basic mechanisms that are controlled by similar metabolic pathway activated genes, there are also important differences. For instance, the rodent cerebral cortex is basically a primary cortex, whereas the primary sensory areas in humans account for a very small surface in the cerebral cortex when compared to the associative and frontal areas that are more extensive. Associative and frontal areas in humans are involved in many neurological disorders, including ASD, attention deficit-hyperactive disorder, and dyslexia, among others. Therefore, an evo-devo approach to neocortical evolution among species is fundamental to understand not only the role of thyroid hormones and environmental thyroid disruptors on evolution, development, and organization of the cerebral cortex in mammals but also their role in neurological diseases associated to thyroid dysfunction.

Keywords: evo-devo, cortical development, autism, thyroid hormones, hypothyroidism

INTRODUCTION

Evolutionary developmental biology (evo-devo) studies the developmental processes of different organisms to determine the ancestral relationships between them and to discover how developmental processes evolved. It addresses the origin and evolution of embryonic development and the modifications of developmental process that produced novel features (Wikipedia, accessed August 2014). Evo-devo teaches us that some fundamental developmental processes are preserved by the evolution among species (1). The evo-devo approach is not only becoming crucial for the modern study of evolution but also it helps in the understanding of morphofunctional alterations in human psychiatric diseases. For instance, autism spectrum disorders (ASD) show abnormal function of cortical areas, such as the frontal or associative neocortices that are minimally present in rodents (2, 3). An approach to the etiologic factors of psychiatric diseases can be inferred by the study of homologous genetic pathways that lead to similar developmental processes in both humans and other mammals. A second issue is that several psychiatric diseases, including ASD, show a wide spectrum of different phenotypes, which are the result of both genetic (nature) and environmental (nurture) factors (4); including among the latter the interaction of comorbid disorders such as hypothyroidism and hypothyroxinemia (5). We begin this review with a summary of thyroid hormone synthesis, transport, and cell actions, which are regulated by a very complex assembly of transporters, deiodinases, receptors, and cofactors. As such, tissues have some control over thyroid hormone action, independent of circulating levels of thyroid hormones. We continue with the analysis of the role of thyroid hormones at different phases of brain development and maturation, focusing our attention on vulnerable periods. These periods occur during gestation and lactation when genetic and environmental factors, which include nutrients and chemical contaminants, interfere with maternal and offspring thyroid health. There is evidence that anatomical characteristics of autistic brains represent defects in processes that occur early in development, in the first half of gestation. Moreover, genomic studies have revealed a catalog of critical genes for these processes that are regulated by thyroid hormones. Finally, recent studies have reported that thyroid hormone deficiency might contribute to increase the number of autism phenotypes, and that disorders associated with hypothyroidism and hypothyroxinemia, such as intellectual impairment, seizures, and anxiety, are comorbid of ASD.

THYROID FUNCTION DURING BRAIN DEVELOPMENT

Thyroid hormones (T4, thyroxine; and T3, 3,5,3′-triiodo-L-thyronine) are synthesized in the thyroid gland and are transported to different tissues and organs where they regulate growth, maturation, and function in many organs and systems of vertebrates. In particular, the mammalian central nervous system (CNS) is an important target of thyroid hormones from fetus to adult. However, the maximal vulnerability of the CNS to thyroid hormone imbalance occurs during the earliest stages of brain development (6–15).

In target cells, thyroid hormones can exert their action at three levels: nuclear and mitochondrial (genomic) and non-genomic (16). Genomic actions include (1) thyroid hormone cell membrane transport, (2) thyroid hormone metabolism (involving its activation/degradation), and (3) binding to nuclear thyroid hormone receptors (TRs, also known as THRs), which are ligand-regulated transcription factors (17–25).

THYROID HORMONE CELL MEMBRANE TRANSPORT

Thyroid hormone cell membrane transport is mediated by four families of transporters: the Na^+/taurocholate cotransporting polypeptide (NTCP), the organic anion transporting polypeptide (OATP), monocarboxylate transporter (MCT), and the heterodimeric amino acid transporter (HAT) (26). From these, Oatp14, Mct8, Mct10, Lat1, and Lat2 have been found to be expressed in the brain (20, 26–33).

THYROID HORMONE METABOLISM (ACTIVATION/DEGRADATION)

Three selenoproteins catalyzing the deiodination of T4 (thyroxine) and T3 (the active form for the genomic action) have been identified: type 1 (D1), type 2 (D2), and type 3 (D3) iodothyronine deiodinases. Only D2 and D3 have been found expressed in the CNS. D2 has been found in the astrocytes and tanycytes [special ependymal cells, Ref. (34)] and mediates the local generation of T3. D3 mediates the degradation of T3 to T2 (diiodothyronine, 3,5-diiodo-L-thyronine) and T4 to rT3 (35–37). In addition to deiodination, iodothyronines are also metabolized by conjugation of the phenolic hydroxyl group with sulfate or glucuronic acid (38).

THYROID HORMONE NUCLEAR RECEPTORS

In the CNS, there are three nuclear TR isoforms with high-affinity to T3: TRα1 (codified by the *THRA* gene), TRβ1, and TRβ2 (codified by the *THRB* gene) (17, 20, 39, 40). TRα1 is the most ubiquitous; it has been detected in the rat brain by embryonic day 12 (E12) and in the human brain by the 10th week of gestation (41–43), regulating the expression of genes involved in the development and maturation of the brain (44), while TRβ1 is mostly expressed in the adult. In addition, N-terminal truncated TRα1 (also known as p43) can serve as a T3-dependent transcription factor that initiates global mitochondrial transcription (16, 45, 46).

Recent studies have shown that thyroid hormone signaling is more diverse and complex than initially concluded. For instance, apart from the canonic role of thyroid hormones mentioned above, novel THRs synthetic ligands might also modulate TRs action, and intra and extracellular signals can affect cell sensitivity to T3 influencing *TRs* gene expression, TRs translation and its transport into the nucleus, and the recruitment of co-activators/-inhibitors (21, 24, 47). Furthermore, thyroid hormones can show non-genomic actions by binding to cell surface or cytoplasmic receptors and by interacting with other signaling pathways (16, 21, 48).

In rodents and humans, almost all T3 found in the fetal cerebral cortex is generated through local deiodination of circulating maternal T4 (13, 49, 50). The fetal dependence on maternal T4 is due (i) to the late development of the fetal thyroid gland (in rodents thyroid function begins by E17–18 and in humans by the 18–20 gestational week) and (ii) to the increased activity of D2 and D3 deiodinases in placenta and fetal tissues (13, 35, 51, 52). As a consequence of the increased activity of deiodinases in the fetus, serum T3 levels are maintained low and the local generation of cerebral T3 from T4 is enhanced (13, 50). To respond to this requirement, there is an estrogen-dependent increase of maternal thyroid function that transiently induces an increase of (i) circulating thyroxine-binding globulin, affecting the T4 extra-thyroidal pool, and of (ii) human chorionic gonadotropin, transiently stimulating thyrocytes (53). This increased maternal thyroid function consequently needs increased iodine intake.

NUTRITIONAL AND ENVIRONMENTAL FACTORS AFFECTING THYROID FUNCTION

Several factors can affect thyroid function during gestation and early postnatal development, including genetic mutations, infections, nutrients, and environmental contaminants. Iodine deficiency from inadequate alimentary habits is the most common cause of maternal and fetal thyroid dysfunction (54–57). In addition, selenium (a component of deiodinases), iron (a component of the prosthetic heme group associated to the thyroperoxidase), and other micronutrients are required for an adequate life-long thyroid function, especially during development and adolescence (58). Moreover, environmental anti-thyroid contaminants are acquiring increased importance (55, 59–67).

THYROID FUNCTION-DISRUPTING CHEMICALS FROM ENVIRONMENTAL CONTAMINANTS

A thyroid function-disrupting chemical is an exogenous chemical, or mixture of chemicals, that can interfere with any aspect of hormone action (67). The mechanisms of action of disrupting chemicals on thyroid function are not fully understood; some may reduce serum T4 without increasing serum TSH while others may interfere with thyroid hormone action at sites other than the thyroid gland without altering serum TSH levels (21, 67). Howdeshell (59) listed synthetic chemicals that interfere with thyroid hormone synthesis, transport, and metabolism. Some are quite specific such as perchlorate salts that block the sodium/iodide symporter (68), but the majority affects several phases of thyroid hormone action. Some thyroid disruptors are consumed in the diet (5, 63); for instance, plant isoflavonoids such as genistein and daidzein from soy inhibit thyroperoxidase that catalyzes iodination and thyroid hormone biosynthesis; thiocyanate from cassava not only blocks iodine uptake by thyroid and mammary glands but also interferes with thyroid peroxidase. Organochlorides (including mostly DDT and its derivative: *p,p′*-DDE, dichlorodiphenyl dichloroethylene; HCB, hexachlorobenzene; PBB, polybrominated biphenyls;

and PCB, polychlorinated biphenyls) interfere with thyroid function acting upon iodine uptake, thyroid peroxidase action, thyroid hormone binding proteins, and thyroid hormone metabolism, resulting in a wide spectrum of thyroid-related syndromes (59, 69). The increased use of nanoparticles in several industrial, consumer, and medical applications has revealed their unique physico-chemical properties. However, *in vitro* and *in vivo* studies have shown that they may have toxic effects on the endocrine system (70). It has been found that Ag-nanoparticles and cadmium telluride-quantum dots alone induced a reduction in the expression TRβ (71).

IODINE DEFICIENCY DISORDERS AND NEURODEVELOPMENTAL DAMAGE

As mentioned before, during gestation, the mother must produce sufficient amounts of thyroid hormones (fundamentally T4) for herself and her fetus. Iodine intake is the principal source of circulating inorganic iodine; therefore, sufficient iodine is critical for the thyroid gland to produce adequate amounts of thyroid hormones (9, 13, 53, 57, 72–77). The fetus also depends on the mother for its iodine supply, as does the neonatal thyroid during lactation (78). To achieve this, expecting mothers need to double the recommended normal daily intake of iodine for non-pregnant women by 250–300 μg/day (79).

Useful food strategies developed to increase iodine intake in iodine-deficient areas include (i) use of iodinated salt in the household, (ii) incorporation of iodine to industrially elaborated foods (i.e., bread, milk, and cheese), and (iii) dietary diversification (i.e., consuming food from iodine-sufficient areas and seafood). Despite these strategies, inadequate iodine intake actually affects a large number of women during pregnancy and lactation, and this situation currently persists even in countries classified as free of iodine deficiency where iodized salt consumption has been promoted for years (79–84).

Iodine deficiency is one of the most frequent causes worldwide of preventable mental retardation in children (85). A wide spectrum of iodine deficiency disorders has been described during gestation and the early postnatal period (<3 years of age), ranging from abortion, stillbirths, congenital anomalies, deafness, cretinism, neurocognitive delay, epilepsy, schizophrenia, ASD, as well as attention deficit hyperactive disorder (ADHD), among others (3, 8, 63, 72, 86–94). In children, the severity of the neurodevelopmental damage caused by iodine deficiency during gestation depends on several factors: (i) the developmental period affected, (ii) its severity, (iii) the deficiency of other nutrients such as selenium and iron, and (iv) the interaction with thyroid function disruptors (9, 10, 14, 57, 58). Epidemiological studies performed in several countries have shown that hypothyroxinemia due to mild iodine deficiency during gestation causes neurological alterations, including low IQ in children (8, 87, 88, 95–99). As mentioned above, iodine deficiency in conjunction to the deficiency of other nutrients and the interaction with thyroid function disruptors will cause a wide spectrum of syndromes associated to thyroid pathologies. In countries with severe iodine and selenium deficiencies, a high incidence of Kashin–Beck osteoarthropathy associated with cretinism has been observed (100). The incidence of myxedematous cretinism increases in countries where severe iodine and selenium deficiency is associated with high intake of thyroid disruptors found in foodstuffs such as cassava, which contain thiocyanate (5, 101). The study of the alterations resulting from nutritional deficiencies in combination with thyroid function disruptors should contribute to our understanding of the multiple syndromes observed in thyroid diseases.

CRITICAL ISSUES OF CEREBRAL CORTEX DEVELOPMENT

For ethical reasons, the role of thyroid hormones on brain development is currently studied using animal models, usually mice and rats. However, although there are basic common developmental principles regulating brain development between mammals, there are also important differences. For instance, the understanding of how different types of neocortex evolved depends on determining not only the numbers and types of cortical areas that exist but also how the internal organization of those areas was modified in the various lines of evolution, including modifications in columnar organization (102). Sexual dimorphism among species also plays an important role, particularly in humans, while in rodents little is known about sex differences between cerebral hemispheres (103). Changes in the organization and size of neocortex also are reflected in the size of cortico-cortical and subcortical projections, in turn affecting target areas (104). Thus, increasing our evo-devo knowledge on neocortical evolution among species (2) will help us to understand not only the role of thyroid hormones and environmental thyroid disruptors on the development, organization, and evolution of the cerebral cortex in mammals (105), but also their role in human associated diseases. The evo-devo considers crucial for the evolution that homologous developmental gene networks are shared among species (1), and it emerges from the relationship between developmental biology and evolution, which in turn are dynamically coupled (106). For instance, basic gene networks involved in symmetric divisions of ventricular neuroblasts during cerebral corticogenesis are common in rodents and humans, while humans evolved by increasing the number of symmetrical divisions, which results in an increased number of cortical columns and therefore an increased cortical surface [see figure 2A by Rakic (2)]. Apart from homology, convergence can also bring solutions for common functional problems. However, little is known on how functional needs have selected different functional networks to generate a similar function between different species, such as the wings of birds and bats (1). Genetic (nature) and environmental (nurture) factors cooperate along time, resulting in differentiation (4). Psychiatric diseases, such as ASD, occur in cerebral areas (e.g., frontal and associative) that are not present in rodents; however, many homologous functional networks, like those involved in radial migration, have been preserved. Furthermore, ASD show a wide spectrum of different phenotypes, resulting in different degrees of morphofunctional alterations and in the concurrence of different comorbid disorders (107). Thyroid hormone deficiency increases comorbidity and the risk of developing ASD (3). For instance, thyroid hormone deficiency during neocorticogenesis results in abnormal development of cortical gamma-aminobutyric acid (GABA)-ergic neurons, which cause altered columnar function in the cerebral cortex and ASD comorbid seizures (108).

The cerebral cortex in all mammalian species, including humans, differs from the development of other organs of the body and even from the rest of the brain. It is a three-dimensional sheet of layers, parallel to the pial surface, mostly composed of projection (or glutamatergic pyramidal) and local neuronal circuits (or glutamatergic and GABAergic interneurons) organized in vertical (or radial) columns that are stereotypically interconnected and share extrinsic connectivity in order to achieve their functions (109). During telencephalic corticogenesis in mammals, including humans, layer I and subplate (deriving from the superficial primordial plexiform layer) are the first cortical layers to appear (110). Subsequently, young cortical neurons begin to migrate radially from the ventricular zone into the superficial cortical plate, adjacent to layer I, following an "inside-out" gradient (111). While in rodents, the neurogenesis of layer I is arrested when radial migration begins, in primates neurogenesis continues during all the periods of corticogenesis (112). Neurons migrate radially to the increasingly distant cortex following the scaffolding of a transient population of radial glial cells (113), in which many signaling pathways – such as reelin, metabolic functions, and gene expression must be involved (114). This phase of corticogenesis is of capital importance because an evo-devo approach of neocortical development and evolution can be explained by the radial unit hypothesis proposed by Rakic (115). As reported by Rakic (2) and mentioned above, increased number of symmetrical divisions will increase the number of functional columns, resulting in increased tangential cortical surface, while that of asymmetrical ones will increase the number of cells per column, resulting in increased cortical thickness [see also figure 2A by Rakic (2)]. The final number of these divisions will depend of apoptotic, anti-apoptotic, or inhibitory factors, and will give rise to either the small lissencephalic cerebrum of rodents or to the larger convoluted cerebrum of humans, as well as to the emergence of new functional areas, such as the prefrontal cortex and associative perisylvian areas (2). The graded expression of transcription factors such as Emx2, Pax6, Coup-Tf1, and Sp8 are implicated in the arealization of the neocortex (116, 117). Deletion or overexpression of these factors results in changes in gene expression, contractions, and expansions in the sizes of cortical fields, and altered patterns of connectivity from the dorsal thalamus (117). Emx2 and Fgf genes share reciprocal functions in regulating cortical patterning; in the frontal cortex, this is accomplished at least in part through controlling the levels of Erm, Er81, Pea3, and Sp8 expression (118, 119). These results support the protomap model (115, 120, 121) because neurons are committed to their areal position at the time of their last cell division (the asymmetrical one) in the proliferative zones in the absence of thalamic afferent inputs, although individual cortical areas may be selectively changed in size during the course of evolution by altered expression signals of their downstream transcription factor signaling mechanisms, as mentioned above (2). In addition, changes in gene expression extrinsic to the neocortex in response to physical stimuli in a particular environmental context might play a crucial role in the formation of domains and areas in the neocortex (117, 122, 123).

In rodents, radially migrating neurons comprise about 80% of the total cortical neurons and will become glutamatergic neurons. The remaining 20% of the cortical neurons migrate tangentially (i.e., parallel to the pial surface) from the ganglionic eminences to their target area and will become local circuit neurons, mostly GABAergic neurons (124–126). In humans, differently from rodents, a subset of neocortical GABAergic neurons [Mash1-positive; a marker for precursors of glutamic acid decarboxylase (GAD)-expressing cells] originates in the ventricular/subventricular zones of the dorsal telencephalon as a distinct neuronal stem cell lineage [Ref. (127, 128); see figure 5 by Rakic (2)]. The identification of the telencephalic origin of local circuit neurons in cerebral cortex of mammals is of capital importance to understand mechanisms operating during primate brain evolution (2, 129, 130) and the pathogenesis of congenital and acquired neurological disorders, such as ASD, related to defects of separate classes of local circuit neurons (131, 132).

In rats, the bulk of neocortical radial migration starts by embryonic day 13 (E13), while the last cohort of cells leaves the ventricular zone by E20 (133). During this process of radial and horizontal migrations, the subplate neurons attract "waiting" afferents from ipsilateral and contralateral cortical areas (including associative and commissural connections), and subcortical connections [including thalamic, nucleus basalis, and monoamine connections (2), see also the figure 2B of this reference]. At the end of this process, neurons and glial cells grow and differentiate, including the loss of juvenile transient connections, to express their mature phenotype, which also contributes to the radial and tangential expansion of the cortex (134). In humans, neocortical development occurs between the 6th and 24th week of gestation (110, 135). The main waves of radial migration in the human neocortex occur during the first half of gestation, with peaks at 11 and 14 weeks of gestational age (110, 135), and mostly before onset of fetal thyroid hormone secretion by the 18th week of gestation (136). This roughly corresponds to waves of cell migration studied in rats (10), which also occur before onset of fetal thyroid hormone secretion, by E17.5–18 (136). Despite the longer development and maturation of the CNS in humans compared with rats, similarities may be established when the onset of fetal thyroid gland secretion is taken as the reference point. However, when comparing the rodent lissencephalic and the primate convoluted mature neocortex, the major differences are found in the tangential rather than in the radial expansion [see figure 1 by Rakic (2)].

EXPERIMENTAL MODELS TO STUDY CORTICAL ALTERATIONS CAUSED BY THYROID HORMONE DEFICIENCY

Several experimental models have been developed to study alterations in the CNS caused by thyroid hormone deficiency. These models can be grouped into (i) genetic mutants, (ii) surgically induced hypothyroidism, (iii) metabolite deficient diets, and (iv) thyroid function disruptor models.

Several genetic models were developed during the last decades to study different forms of developmental and postnatal hypothyroidism, such as congenital hypothyroidism (137). Genetic models can be classified into two main groups: (1) mutations affecting thyroid gland development and function, and (2) mutations affecting thyroid hormone sensitivity, which includes thyroid hormone cell membrane transport, metabolism, and action (25). The first group includes mutations of the TSH receptor (*hyt*$^{-/-}$ mice) (138) and agenesis or functional impairment of thyrocytes

(*TTF1*$^{-/-}$, *TTF2*$^{-/-}$, and *Pax8*$^{-/-}$ mice) (139). The second group includes thyroid hormone transporters mutants such as *Mct8*$^{-/y}$ (140, 141), *Mct8*$^{-/-}$ (142), and *Lat2*$^{-/-}$ (143). These mutant mice have provided new data to understand thyroid hormone transport in the cell membrane and clarified the physiopathology of the Allan–Herndon–Dudley syndrome, which is caused by MCT8 defect (141, 144–146). Thyroid hormone metabolism in the brain has been studied using different mutant mice affecting D2 and D3 expression (*Dio2*$^{-/-}$, Dio3$^{-/-}$, and *Dio2*$^{-/-}$*Mct8*$^{-/y}$ mice) (147–149). Important genes associated to cortical development are affected in Dio mutants. In particular, the neuronal genes *Gls2* (glutaminase 2), *Nefh* and *Nefm* (heavy and medium neurofilament polypeptide), *Sema7a* (semaphorin 7A), *Shh* (sonic hedgehog), *Col6a1* and *Col6a2* (type VI α1 and α2 collagen), as well as *Slc1a3* (glial high-affinity glutamate transporter) and Itga7 (integrin α7), among others, found in glial cells (148). Mutations of the *TR* gene include *TR*α$^{-/-}$, *TR*β$^{-/-}$, and *TR*α$^{-/-}$β$^{-/-}$ mice, as well as *TR*α and *TR*β knock-in mutations (23, 150). Mutations of *TR*β gene are associated to the Refetoff syndrome (151, 152). A classification of these mutations and their associated syndromes of impaired sensitivity to thyroid hormone has been recently published (25).

The most common models are based on the administration of anti-thyroid drugs interfering either with the thyrocytes iodine uptake by inhibiting the sodium/iodine symporter (e.g., potassium perchlorate and thiocyanate) or with the iodination of thyroglobulin by thionamide and thiourylene drugs such as propylthiouracil (PTU) and methimazole (MMI) (153–155). In addition, PTU (and less MMI) partially inhibits iodothyronine deiodinases affecting the peripheral deiodination of T4 (154, 156). Anti-thyroid treatments result in maternal, fetal, and neonate hypothyroidism of greater or lesser severity (157). MMI treatment was also used experimentally to induce mild and transient maternal hypothyroxinemia at the onset (E12) of fetal neocorticogenesis (158, 159). Models for iodine deficiency during gestation include monkeys (160), sheep (161), and rats (162, 163). These studies have shown changes in the cerebellum with reduction in weight and cell number, and delayed maturation. The influence of iodine deficiency on neocortical development has been studied in rats that are fed a low iodine diet during pregnancy (163–166).

Alternatively, surgical thyroidectomy can be used to induce hypothyroidism (167, 168), when performed in pregnant dams it causes maternal but neither fetal nor neonate hypothyroidism. Recently, late maternal hypothyroidism (LMH) during gestation has been used as a model to study the role of maternal thyroid hormones from the onset of fetal thyroid function (169).

ALTERATIONS IN CORTICAL DEVELOPMENT CAUSED BY THYROID HORMONE DEFICIENCIES

GENES REGULATED BY THYROID HORMONES INVOLVED IN BRAIN DEVELOPMENT

Fundamental genes involved in brain development are regulated by thyroid hormones. The irreversibility and importance of damage will depend on when, where, and how the alterations of gene expression occur (10, 20). Early studies showed that maternal thyroid hormones regulate gene expression in fetal development modulating the expression of *NSP* and *Oct-1* genes; T4 injections produced rapid, transient, and selective effects on gene expression in the fetal brain (170). Additional genes regulated by maternal thyroid hormones included *Nrgn* (neurogranin, also known as RC3), found to be significantly decreased (171), as well as reelin, apolipoprotein E receptor 2 (ApoER2; a reelin receptor involved in the migration young neocortical neurons), very-low-density lipoprotein receptor (VLDLR; a reelin receptor that mediates the stop signal), integrin genes, and genes involved in the downstream phosphorylation of Dab1 (very-low-density lipoprotein receptor 1) (172, 173). cDNA microarray studies have shown a number of genes to be transcriptionally or functionally modulated by T3; most of these are involved in cell division, migration, growth, connectivity, and function of neural cells. Using rat pituitary GC cell line, Miller et al. (174) showed that 358 out of 4,400 genes were regulated by T3; and, in a recent study, Morte et al. (44) found 552 out of 14,209 genes regulated by fetal and maternal thyroid hormones at the end of gestation in rats. The function of some of these genes is unknown but most of them are involved in the regulation of key pathways for the development of the cerebral cortex in rodents and humans. **Tables 1–6** list some of the most relevant T3-regulated genes at the transcriptional level. Among those of relevant importance for the development of cortical connections are *Nefh*, *Nefl*, and *Nefm* (coding neurofilament proteins); *Slit1*, *Slit2*, *Nos1*, *Camk4*, and *Creb1* (involved in bifurcation and growth of neural processes); *Sema3B*, *Slit1*, and *Slit2* (guiding axons); and *Slc17a7* (coding vesicular glutamate transporter 1; VGluT1). T3 action on the regulation of the Camk4/Creb pathway and downstream targets (175) in neurons of the CNS is highly relevant since Camk4 has not been found expressed in glial cells (169, 176, 177). Camk4 is directly induced by T3 at the transcriptional level (44), and phosphorylates Creb. Many of the genes under thyroid hormone control contain Creb binding sites in their promoter region (149). On the other hand, Camk4 regulates the transcriptional activity of the TR, which might be due to direct phosphorylation of co-activators or by changing the equilibrium between the co-activators and the silencing mediator for retinoid and thyroid hormone receptors (SMRT) (178, 179). Camk4/Creb pathway and downstream targets are involved in processes such as neurogenesis, biosynthesis, and assembly of cytoskeleton, cell movement and migration, neurite development and maturation, synaptic plasticity, and neurotransmission (44, 180). In humans, Camk4/Creb pathway is involved in psychiatric disorders (181–183). There is a strong evidence for the action of Camk4/Creb pathway in the expression of *FMR1* gene, encoding fragile X mental retardation protein (FMRP) (184, 185). Lack of FMRP causes fragile X syndrome, which is the most common cause of inherited mental retardation and ASD (186, 187). In addition, brain-derived neurotrophic factor (BDNF)/Erk signaling modulates FMRP function, affecting neuronal proliferation and differentiation in the cerebral cortex [Ref. (188, 189); **Table 5**].

ALTERED NEUROGENESIS AND MIGRATION DURING CORTICOGENESIS

Indirect observations based on the cell density estimates and brain size measurements suggested a reduced number of cells in the neocortex of developmentally hypothyroid rats (190). Neural progenitors in the ventricular zone of mouse telencephalon express TRα1, Mct8 transporters, and deiodinases, and maternal hypothyroidism

Table 1 | Significant T3-regulated genes at the transcriptional level found in the cerebral cortex of rodents, involved in cell division and differentiation: relationship with ASD.

Symbol[a]	Protein	Process	Alteration/disease
ADCYAP1R1	Adenylate cyclase-activating polypeptide receptor (PAC1)	Signaling pathway	Decreased second messenger
CASP3	Caspase 3	Protease	Apoptosis. Alzheimer's disease
CCND1	G1/S-specific cyclin-D1	Interact with tumor suppressor protein Rb	Abnormal cell cycle G1/S transition
CNN1	Calporin (actin binding protein; fimbrin type)	Actin associated protein	Abnormal cohesion between parental centrioles
CREB1	cAMP-responsive element binding protein 1	Transcription factor	Altered development. ASD
CREM	cAMP-responsive element modulator	Transcription factor modulating CREB	Altered development. ASD
CTNNB1	β-catenin	Regulates the coordination of cell–cell adhesion and gene transcription	Altered asymmetric cell division, epithelial-to-mesenchymal transition. ASD
DYRK1A	Dual specificity tyrosine-phosphorylation-regulated kinase 1A	Nuclear signaling	Abnormal cell proliferation and may be involved in brain development. ASD
GNB1L	Guanine nucleotide-binding protein subunit β-like protein 1	Six WD40 repeat-containing protein	Abnormal cell cycle progression. Schizophrenia. ASD
FLT1	Vascular endothelial growth factor receptor 1	Protein kinase	Abnormal control of cell proliferation and differentiation. ASD
HIST1H1T	Histone H1t	Compaction of chromatin	Abnormal cell cycle and differentiation
HSD11B2	Corticosteroid 11-β-dehydrogenase isozyme 2	Hydrolysis of cortisol	Cortisol induction of growth-inhibition and/or pro-apoptosis embryonic development
MAPK1	Mitogen-activated protein kinase 1 (ERK2)	CREB1 phosphorylation signaling pathway	ASD
RGS3	Regulator of G-protein signaling 3	Ephrin-B signaling pathway	Early cell cycle exit and precocious differentiation

[a] *Bold shows T3-regulated genes that have been found to be abnormally expressed in autistic humans. Other genes found in autistic humans not regulated by T3 at the transcriptional level have not been included.*

reduces the cell cycle length of these progenitors (191). Since in hypothyroid fetuses the bulk of the neocortical BrdU-labeling occurs between E12 and E19 as in control rats (192), the data by Mohan et al. (191) clearly indicated that the total neuronal progenitor number is reduced in the cerebral cortex. Using ^{3}H-thymidine labeling, a significant reduction was observed in cell acquisition in the granular layer of the hippocampal dentate gyrus in postnatal PTU treated pups (193). These authors also observed that the radial migration of newly generated hippocampal granular cells could be arrested and that the decreased number of labeled cells in the granular layer might result from deficient migration rather that decreased mitotic activity. Several genes involved in cell cycle regulation during neurogenesis have been found to be regulated by T3 [Ref. (44); **Table 1**]. CCND1 (G1/S-specific cyclin-D1) is downregulated by T3, resulting in an abnormal cell cycle progression (194). In addition, T3-regulated regulator of G-protein signaling 3 (RGS3) plays a key role in ephrin-B signaling, controlling cell cycle exit, and differentiation of neural progenitors (195), and dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) is involved in the control of cell proliferation in ASD, causing arrested brain growth (196). Other T3-regulated genes such as CNN1 (calporin), which is an actin associated protein, also exerts a control of cell cycle during neurogenesis (197). Recently, it has been found that developmental mild and severe hypothyroxinemia and MMI-induced hypothyroidism alters Shh signaling pathway in the cerebellar granule cell precursors, resulting in downregulation of D1 and D2 cyclins, of E2F1 expression, and in reduced cell proliferation (198). However, it still remains unclear to what extent thyroid hormones affect symmetrical and asymmetrical divisions of neocortical progenitor cells. Studies on the mouse barrel cortex (192) suggest that both symmetrical and asymmetrical divisions are altered in hypothyroid rats, because the tangential area of the posteromedial barrel subfield stained with cytochrome oxidase (resulting from symmetrical divisions) and the thickness of the barrel cortex (resulting from asymmetrical divisions) are reduced by 27 (**Figures 1A–D**) and 12.5% (**Figure 1E**), respectively, in hypothyroid rats. Nevertheless, from these data we could argue that most likely the symmetrical divisions are comparatively more affected in hypothyroid rats. The reduced thickness of the cortex in hypothyroid rats could be explained by a reduction

Table 2 | Significant T3-regulated genes at the transcriptional level found in the cerebral cortex of rodents, involved in cytoskeleton organization and cell migration: relationship with ASD.

Symbol[a]	Protein	Process	Alteration/disease
APOER2	Apolipoprotein E receptor 2 (Lrp8)	Reelin signaling pathway	Alzheimer, major depressive disorder
CALR	Calreticulin	Endoplasmic reticulum calcium-binding protein	Abnormal calcium storage in the hippocampus. Alzheimer's disease
CREB1	cAMP-responsive element binding protein 1	Transcription factor	Altered development. ASD
CREM	cAMP-responsive element modulator	Transcription factor modulating CREB	Altered development. ASD
CTSS	Cathepsin S	Protease	Abnormal microglial function
DAB1	Disabled-1	Reelin signaling pathway	Abnormal migration. Alzheimer's disease, temporal lobe epilepsy. ASD
DYNLL1	Dynein light chain 1, cytoplasmic	Microtubule associated protein	Abnormal intracellular transport and motility
FMOD	Fibromodulin	Proteoglycan that sequesters TGF-β into the extracellular matrix	Abnormal regulation of proliferation and differentiation of hippocampal granule neurons
FN1	Fibronectin	Extracellular matrix protein	Abnormal cell adhesion, growth, migration, and differentiation. ASD
GNAS	G-protein α subunit (Gs-α)	Signaling pathway	ASD. ADHD
HSPD1	Chaperonin (HSP60)	Chaperone	Prevent traumatic brain injury
MAPK1	Mitogen-activated protein kinase 1 (ERK2)	CREB phosphorylation signaling pathway	ASD
NEFH, ***NEFM***, ***NEFL***	Neurofilament protein (heavy, medium, and light)	Intermediate filaments	Abnormal neuronal cytoskeleton. ASD
NOV	Nephroblastoma overexpressed	Extracellular matrix protein that binds to integrin receptors	Abnormal cell adhesion, migration, proliferation, differentiation, and survival
OPCML	Opioid-binding protein/cell adhesion molecule	Cell adhesion molecule	Abnormal proliferation and growth of cortical astrocytes
PAFAH1B1	Platelet-activating factor acetylhydrolase IB subunit α (Lis1)	Interact with dynein and VLDLR	Lissencephaly. ASD
RELN	Reelin	Extracellular matrix protein	Abnormal migration. Alzheimer's disease, temporal lobe epilepsy. ASD
SERPINH1	Heat shock protein 47	Chaperone	Abnormal collagen binding. ASD
SLIT1, *SLIT2*	Slit homolog 1 and 2 proteins	Extracellular matrix protein. Chemorepulsive signal	Abnormal axon guidance. Abnormal angiogenesis
TGFB2	Transforming growth factor-β 2	Extracellular matrix protein	Abnormal regulation of proliferation and differentiation of hippocampal granule neurons
TPM1	Tropomyosin α-1 chain	Actin associated protein	Abnormal neuronal cytoskeleton
VLDLR	Very-low-density-lipoprotein receptor	Reelin signaling pathway	Abnormal migration. Alzheimer's disease, temporal lobe epilepsy. ASD

[a] *Bold shows T3-regulated genes that have been found to be abnormally expressed in autistic humans. Other genes found in autistic humans not regulated by T3 at the transcriptional level have not been included.*

Table 3 | Significant T3-regulated genes at the transcriptional level found in the cerebral cortex of rodents, involved in neurite growth, guidance, branching, and maturation: relationship with ASD.

Symbol[a]	Protein	Process	Alteration/disease
ANK3	Ankyrin-3	Cytosol protein that interacts with voltage-gated sodium channels and cytoskeletal proteins	Abnormal clustering of voltage-gated sodium channels at the axon hillock and node of Ranvier abnormal action potential firing. ASD
ARX	Aristaless-related homeobox	Transcription factor	X-linked intellectual disability, epilepsy, lissencephaly, agenesis of the corpus callosum. ASD
BDNF	Brain-derived neurotrophic factor	Extracellular signal	Abnormal synaptic structure, function, and plasticity. Fragile X syndrome. ASD
CAMK4	Calcium/calmodulin-dependent protein kinase type IV	CREB phosphorylation signaling pathway	ASD
CHN1	Chimerin 1 (GTPase-activating protein)	Signal transduction	Abnormal axon pruning
CNTN4	Contactin-4	Cell adhesion molecule	Abnormal connectivity in the developing nervous system. ASD
CREB1	cAMP-responsive element binding protein 1	Transcription factor	Altered development. ASD
CREM	cAMP-responsive element modulator	Transcription factor modulating CREB	Altered development. ASD
FLT1	Vascular endothelial growth factor receptor 1	Protein kinase. Signal transduction	Abnormal control of cell proliferation and differentiation. ASD
FN1	Fibronectin	Extracellular matrix protein	Abnormal cell adhesion, growth, migration, and differentiation. ASD
HAP1	Huntingtin-associated protein 1	Interacts with huntingtin and cytoskeletal proteins	Abnormal vesicular trafficking and organelle transport
KLF9	Kruppel-like factor 9	Transcription factor	Altered development of neurons
MAPK1	Mitogen-activated protein kinase 1 (ERK2)	CREB phosphorylation signaling pathway	ASD
NEFH, ***NEFM***, ***NEFL***	Neurofilament protein (heavy, medium, and light chains)	Intermediate filaments	Abnormal neuronal cytoskeleton. ASD
NOS1	Nitric oxide synthase 1	Neurotransmitter, signaling pathway	Abnormal signaling pathway. Neuroglial inflammation. ASD
PLXNA2,3	Plexin-A2	Semaphorin co-receptor	Abnormal axon guidance. Schizophrenia, anxiety
SEMA3B	Semaphorin-3B	Signal transduction	Abnormal axon guidance
SLIT1, *SLIT2*	Slit homolog 1 and 2 proteins	Extracellular matrix protein. Chemorepulsive signal	Abnormal axon guidance. Abnormal angiogenesis
TGFB2	Transforming growth factor-β 2	Extracellular signaling protein	Abnormal regulation of proliferation and differentiation of hippocampal granule neurons

[a] *Bold shows T3-regulated genes that have been found to be abnormally expressed in autistic humans. Other genes found in autistic humans not regulated by T3 at the transcriptional level have not been included.*

of the columnar neuropile more than by a reduction in the cellular components of the columns. T3-regulated *CASP3* (caspase 3) and *CTNNB1* (β-catenin) genes are crucial for cerebral cortex expansion (**Table 1**). Experimental studies using caspase 3 and 9 KO mice (lacking apoptotic signals) (2, 199) and transgenic mice expressing β-catenin (which increases the number of precursor cells) lead to an abnormally convoluted mouse cortex (200). Caspase 3 pathway is downregulated in the cerebral and cerebellar cortices of hypothyroxinemic and hypothyroid rats (201, 202), while β-catenin is T3-downregulated in rat pituitary cultured cells (174). These data show that thyroid hormone deficiency alters the tangential and radial organization of the cortex and might have

Table 4 | Significant T3-regulated genes at the transcriptional level found in the cerebral cortex of rodents, involved in synaptogenesis and plasticity: relationship with ASD.

Symbol[a]	Protein	Process	Alteration/disease
ANXA6	Annexin A6	Calcium-binding protein	Abnormal vesicle aggregation and fusion in the hippocampal neuron's axon initial segment
ATP2B2	$Ca^{(2+)}$-ATPase	Plasma membrane calcium-ATPase	Abnormal translocation of calcium to the endoplasmic reticulum in hippocampal neurons. ASD
BDNF	Brain-derived neurotrophic factor	Synaptic structure, function, and plasticity. fragile X syndrome autism	Abnormal synaptic structure, function, and plasticity. Fragile X syndrome. ASD
CAMK4	Calcium/calmodulin-dependent protein kinase type IV	CREB phosphorylation signaling pathway	ASD
CNTN4	Contactin-4	Cell adhesion molecule	Abnormal connectivity in the developing nervous system. ASD
CREB1	cAMP-responsive element binding protein 1	Transcription factor	Altered development. ASD
CREM	cAMP-responsive element modulator	Transcription factor modulating CREB	Altered development. ASD
EXOC7	Exocyst complex component 7	Rho3 signaling	Abnormal cell polarity, regulation of actin polarity and transport of exocytic vesicles
HAP1	Huntingtin-associated protein 1	Interacts with huntingtin and cytoskeletal proteins	Abnormal vesicular trafficking and organelle transport
HRH3	Histamine H3 receptors	Signal transduction	Abnormal presynaptic inhibition of neurotransmitter release
MAPK1	Mitogen-activated protein kinase 1 (ERK2)	CREB phosphorylation signaling pathway	ASD
NR4A1	Nuclear receptor related 1 protein (NURR77)	Transcription factor	Abnormal synaptic plasticity in the hippocampus. Altered long-term potentiation. Schizophrenia
NRGN	Neurogranin	Calmodulin-binding protein. Component of postsynaptic density	Abnormal synaptic plasticity and long-term potentiation. Schizophrenia. ASD
PAFAH1B1	Platelet-activating factor acetylhydrolase IB subunit α (Lis1)	Interacts with dynein and VLDLR	Lissencephaly. ASD
PICALM	Phosphatidylinositol binding clathrin assembly protein	Coated vesicles	Abnormal coated vesicles. Alzheimer's disease
SLIT1, *SLIT2*	Slit homolog 1 and 2 proteins	Extracellular matrix protein. Chemorepulsive signal	Abnormal axon guidance. Abnormal angiogenesis
SNAP23	Synaptosomal-associated protein 23	SNARE associated protein	Abnormal exocitosis
SNX16	Sorting nexin 16	Membrane associated protein	Protein sorting
SQSTM1	Sequestosome-1	Ubiquitin binding protein	Abnormal regulation of the nuclear factor kappa-B (NF-κB) signaling pathway
SYT2	Synaptotagmin-2	Synaptic vesicles docking	Abnormal exocitosis
SYTL5	Synaptotagmin-like protein 5	Synaptic vesicles docking. Marker for parvalbumin immunoreactive buttons	Abnormal exocitosis
TGFB2	Transforming growth factor-β 2	Extracellular signaling protein	Abnormal regulation of proliferation and differentiation of hippocampal granule neurons
VAMP4	Vesicle-associated membrane protein 4 (synaptobrevin)	Synaptic vesicles docking	Abnormal exocitosis

[a] *Bold shows T3-regulated genes that have been found to be abnormally expressed in autistic humans. Other genes found in autistic humans not regulated by T3 at the transcriptional level have not been included.*

Table 5 | Significant T3-regulated genes at the transcriptional level found in the cerebral cortex of rodents, involved in neurotransmission: relationship with ASD.

Symbol[a]	Protein	Process	Alteration/disease
ADCYAP1R1	Adenylate cyclase-activating polypeptide receptor (PAC1)	Signaling pathway	Decreased second messenger
CACNG8	Calcium channel, voltage-dependent, γ subunit 8	Transmembrane AMPA receptor regulatory protein (TARP)	Altered long-term potentiation
CAMK4	Calcium/calmodulin-dependent protein kinase type IV	CREB phosphorylation signaling pathway	ASD
CREB1	cAMP-responsive element binding protein 1	Transcription factor	Altered development. ASD
CREM	cAMP-responsive element modulator	Transcription factor modulating CREB	Altered development. ASD
HAP1	Huntingtin-associated protein 1	Interacts with huntingtin and cytoskeletal proteins	Abnormal vesicular trafficking and organelle transport
HOMER1	Homer protein homolog 1	Major component of postsynaptic density	Abnormal synaptic plasticity and long-term potentiation. ASD
HRH3	Histamine H3 receptors	Signal transduction	Abnormal presynaptic inhibition of neurotransmitter release
KCNC1	Potassium voltage-gated channel subfamily C member 1	Membrane channel	Abnormal repolarization of cortical interneurons
KCNJ10	ATP-sensitive inward rectifier potassium channel 10	Membrane channel	Abnormal repolarization. Epilepsy, ataxia, and deafness. ASD
KCNK2	Potassium channel subfamily K member 2 (TREK1)	Membrane channel	Abnormal neuroprotection against epilepsy and brain and spinal cord ischemia
KCNS2	Potassium voltage-gated channel subfamily S member 2	Membrane channel	Abnormal repolarization
KCNT2	Potassium channel subfamily T, member 2	Membrane channel	Abnormal repolarization epilepsy, Alzheimer disease
MAPK1	Mitogen-activated protein kinase 1 (ERK2)	CREB phosphorylation signaling pathway	ASD
NRGN	Neurogranin	Calmodulin-binding protein. component of postsynaptic density	Abnormal synaptic plasticity and long-term potentiation. Schizophrenia. ASD
NTS	Neurotensin	Neuropeptide	Abnormal modulation of dopamine signaling. ASD
PACSIN2	Protein kinase C and casein kinase substrate in neurons protein 2	Binding to endocytic proteins	Arrested endocytosis
PAFAH1B1	Platelet-activating factor acetylhydrolase IB subunit α (Lis1)	Interacts with dynein and VLDLR	Abnormal signaling. Lissencephaly. ASD
SLC17A7	Vesicular glutamate transporter 1 (VGLUT1)	Synaptic vesicle membrane protein	Abnormal neurotransmission neuropsychiatric disorders. ADHD, and schizophrenia. ASD

[a] *Bold shows T3-regulated genes that have been found to be abnormally expressed in autistic humans. Other genes found in autistic humans not regulated by T3 at the transcriptional level have not been included.*

contributed in the evolutionary elaboration of radial columns, modulating both cortical surface and thickness.

Altered T3-regulated opioid-binding protein/cell adhesion molecule (OPCML; also known as OBCAM) expression affects radial glia function and its transdifferentiation to astrocytes [Ref. (203); **Table 2**]. In agreement, impaired maturation of radial glia was observed in the hippocampus of pups born to chronic hypothyroxinemic rats (163) and in the neocortex of

Table 6 | Significant T3-regulated genes at the transcriptional level found in the cerebral cortex of rodents, involved in memory and behavior: relationship with ASD.

Symbol[a]	Protein	Process	Alteration/disease
ADCY8	Adenylate cyclase type 8	G-protein associated enzyme	Abnormal cAMP signaling
ADRBK2	β-adrenergic receptor kinase 2 (GRK3)	G-protein-coupled receptor kinase 3	Abnormal dopamine metabolism. Schizophrenia and bipolar disorder
CALB1	Calbindin-D28k	Calcium-binding protein	Abnormal synaptic plasticity and long-term potentiation. ASD
CAMK4	Calcium/calmodulin-dependent protein kinase type IV	CREB phosphorylation signaling pathway	ASD
CREB1	cAMP-responsive element binding protein 1	Transcription factor	Altered development. ASD
CREM	cAMP-responsive element modulator	Transcription factor modulating CREB	Altered development. ASD
DBP	D site of albumin promoter (albumin D-box) binding protein	Transcription factor	Abnormal spatial learning and enhanced susceptibility to kainate-induced seizures. Epilepsy, schizophrenia, and bipolar disorder
GRK5	G-protein-coupled receptor kinase 5	Signal transduction	Memory impairment. Alzheimer's disease
HOMER1	Homer protein homolog 1	Major component of postsynaptic density	Abnormal synaptic plasticity and long-term potentiation. ASD
HTR7	5-HT7 receptor	Neuroreceptor	Abnormal learning and memory. Neuropsychiatric disorders. ASD
MAPK1	Mitogen-activated protein kinase 1 (ERK2)	CREB phosphorylation signaling pathway	ASD
NOS1	Nitric oxide synthase 1	Neurotransmitter, signaling pathway	Abnormal signaling pathway. ASD
NR4A1	Nuclear receptor related 1 protein (NURR77)	Transcription factor	Abnormal synaptic plasticity in the hippocampus. Altered long-term potentiation. Schizophrenia
NTS	Neurotensin	Neuropeptide	Abnormal modulation of dopamine signaling. ASD
PVALB	Parvalbumin	Calcium-binding protein	Alzheimer's disease and nervous system disorders. ASD

[a] *Bold shows T3-regulated genes that have been found to be abnormally expressed in autistic humans. Other genes found in autistic humans not regulated by T3 at the transcriptional level have not been included.*

developmentally hypothyroid pups (204). Abnormal radial migration in the neocortex of developmentally hypothyroid rats was first described in the auditory cortex by combining BrdU and tracer labeling (205). As a result, the radial positioning of migrating neurons was altered, including abnormally located heterotopic neurons in the subcortical white matter (192, 205) (**Figure 2C**) and corpus callosum (206). Also, altered neuronal migration in the neocortex and hippocampus has been confirmed in hypothyroxinemic rats (158, 159, 164) (**Figures 2A–C**). Apart from TRs, other nuclear receptors are involved in radial glia maturation and radial migration in the neocortex such as the liver X receptor β (LXRβ) that also regulates the expression of ApoER2 receptor (207). *LXRβ*$^{-/-}$ mice showed altered cortical migration of later-born neurons (208) and delayed transdifferentiation of radial glial cells into astrocytes (209). Interestingly, LXRs bind to the same response element on DNA as TRs and sometimes regulate the same genes (150, 210). In fact, it has been shown recently that TRα compensates for the lack of LXRβ in cortical development, and a reciprocal compensatory action can also be hypothesized (207).

Heterotopic cells in the external granular layer of the cerebellar cortex have also been observed (212), as well as in *Mct8*$^{-/y}$ mice (213). The stunted migration of cerebellar cells found in previous studies (6, 214, 215) and in TRα1 mutant mice (212, 213) suggests that thyroid hormones interfere with different mechanisms involved in the migration of cortical and cerebellar neurons. Cortical neurons retain most of their migratory capacity as can be observed either in studies combining BrdU and tracer labeling (205) or using organotypic cultures (**Figure 2D**) (159). In the latter, it was found that cells from transient hypothyroid medial ganglionic eminence explants migrate as well as cells from control explants when they were placed on normal host cortex; and reversely, both control and transient hypothyroid median

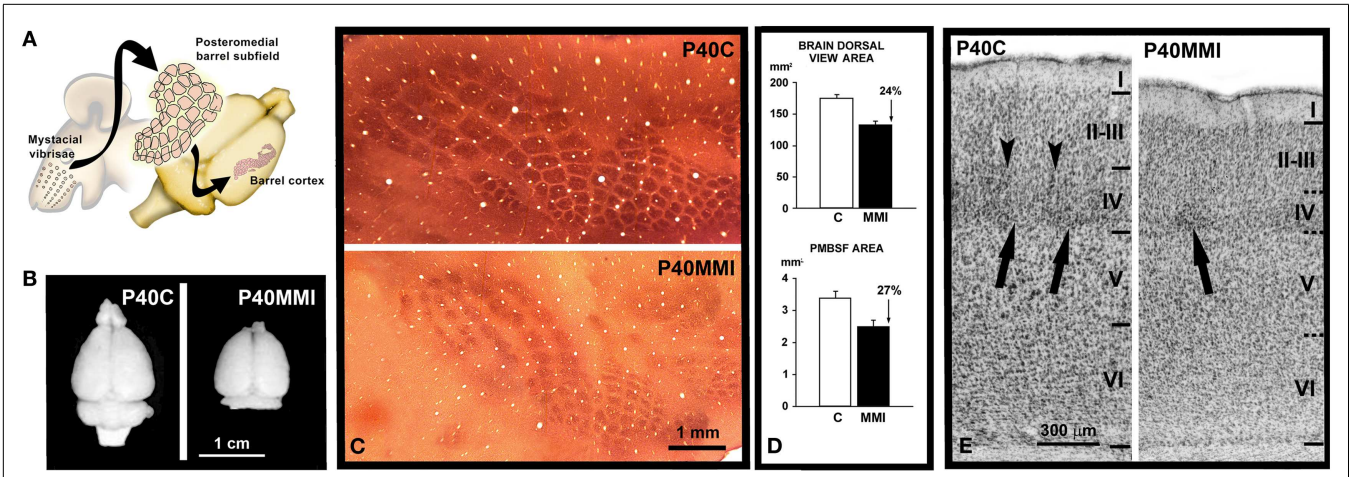

FIGURE 1 | Reduced development of cortical maps in developmental hypothyroidism. **(A)** Cartoon showing the posteromedial barrel subfield of the primary somatosensory cortex in the brain of a rat. Note the correspondence between mysticial vibrises and the barrels of the posteromedial barrel subfield. **(B)** Brain dorsal views at P40 of control (C) and MMI pups. **(C)** Computer reconstruction from photomicrographs of serial tangential sections through layer IV, showing cytochrome oxidase labeling in the barrel cortex of normal and hypothyroid rats. Note the reduced tangential extension of the cytochrome oxidase labeling in hypothyroid with respect to normal rats. **(D)** Area measurements in normal and hypothyroid rats. The dorsal view brain area was, on average, 24% smaller in hypothyroid rats (upper). A similar reduction (on average, 27%) was observed in the PMBSF tangential area (lower). **(E)** Photomicrographs of cresyl violet stained coronal sections showing the cytoarchitecture of the barrel cortex of the primary somatosensory cortex at P40 in control (C) and transient MMI treated pups (MMI treatment begun at E12 and finished at E15). Borders between layers (horizontal lines) are clear-cut in C whereas they are more blurred in MMI12 pups. In layer IV of C and dMMI pups, barrels (arrow) are normal and well-defined and demarcated by septae (arrowheads). In contrast, barrels in layer IV of MMI1 pups are not seen. In developmentally hypothyroid pups there is a 10–15% reduction in the cortical thickness of MMI pups compared to controls. **(A)** Modified from Berbel and Morreale de Escobar (57). **(C,D)** Modified from Berbel et al. (192). **(E)** Modified from Ausó et al. (158).

ganglionic eminence cells showed altered latero-medial migration when placed on transient hypothyroid host cortex, which suggests that in the transient hypothyroid cortex the expression of chemoattractive/-repulsive/-stop signals and/or of their receptors [see review in Ref. (126)] is altered. In fact, some of them, such as Slit1, Slit2, and Sema3B, are regulated by thyroid hormones [Ref. (44); **Table 2**].

ABNORMAL CORTICAL CYTOARCHITECTURE AND CONNECTIVITY

Blurred neocortical layering can be assessed in the rodent somatosensory barrel cortex owing to the characteristic cytoarchitecture of layer IV (192, 216). The parvalbumin immunostaining pattern in hypothyroid rats is severely altered in the neocortex (211, 217, 218) (**Figure 2E**) and hippocampus (217, 219). Interestingly, parvalbumin positive neurons (i.e., GABAergic chandelier and basket neurons that migrate tangentially from the medial ganglionic eminence) also exhibit altered tangential migration in the transient hypothyroxinemic cortex (159). The decreased chandelier and basket parvalbumin immunoreactive terminals in the neocortex (211, 217) and hippocampus (217) will affect the inhibitory control of glutamatergic neurons (220) and might explain the high incidence of audiogenic seizures reported in hypothyroid rats (221) and in the pups of mild and transient hypothyroxinemic pregnant rats (158) (**Figure 2F**).

Early postnatal hypothyroidism affects the growth of dendrites in both the cerebral (193, 222) and cerebellar (223) cortices. Qualitative and quantitative ultrastructural studies of the cerebellar molecular layer in rats show that the retardation in synaptogenesis between Purkinje cell dendritic spines and parallel fibers was associated to hypoplasia of Purkinje cell dendrites and to the retarded development of parallel fibers (223). In the neocortex, it has been found that β-catenin is downexpressed in the dentate gyrus of postnatal hypothyroid rats (165) and the Wnt/β-catenin signaling plays a crucial role for the growth and branching of dendrites (224). Developmental hypothyroidism affects maturation of commissural axons (225–228). In adult hypothyroid rats, the number of myelinated axons was 76 and 66%, respectively, in both the anterior commissure and the corpus callosum compared to controls (228); also, the maturation of cytoskeletal components was altered (226, 229) and the growth of axon caliber was arrested (225, 228). Development and maturation of oligodendrocytes in the forebrain commissures of hypothyroid rats may also be affected. In fact, cortical expression of myelin-associated glycoprotein, proteolipid protein, and myelin basic protein in oligodendrocytes is strongly reduced (230).

Callosal-projecting neurons were found mostly in infragranular layers of the auditory cortex of developing (205) and adult hypothyroid rats (216). In addition to altered radial distribution, the total number of callosal neurons was increased in auditory (216) and visual (226) cortices, and in cortical projecting neurons such as in the occipito-spinal connections (231), revealing maintenance of exuberant projections in hypothyroid rats. Interestingly, in the hypothyroid MMI model, the heterotopic white matter neurons, in particular, the early BrdU-labeled ones normally destined for the subplate, could provide a target to the transient callosal axons as they might in normal development (134, 232).

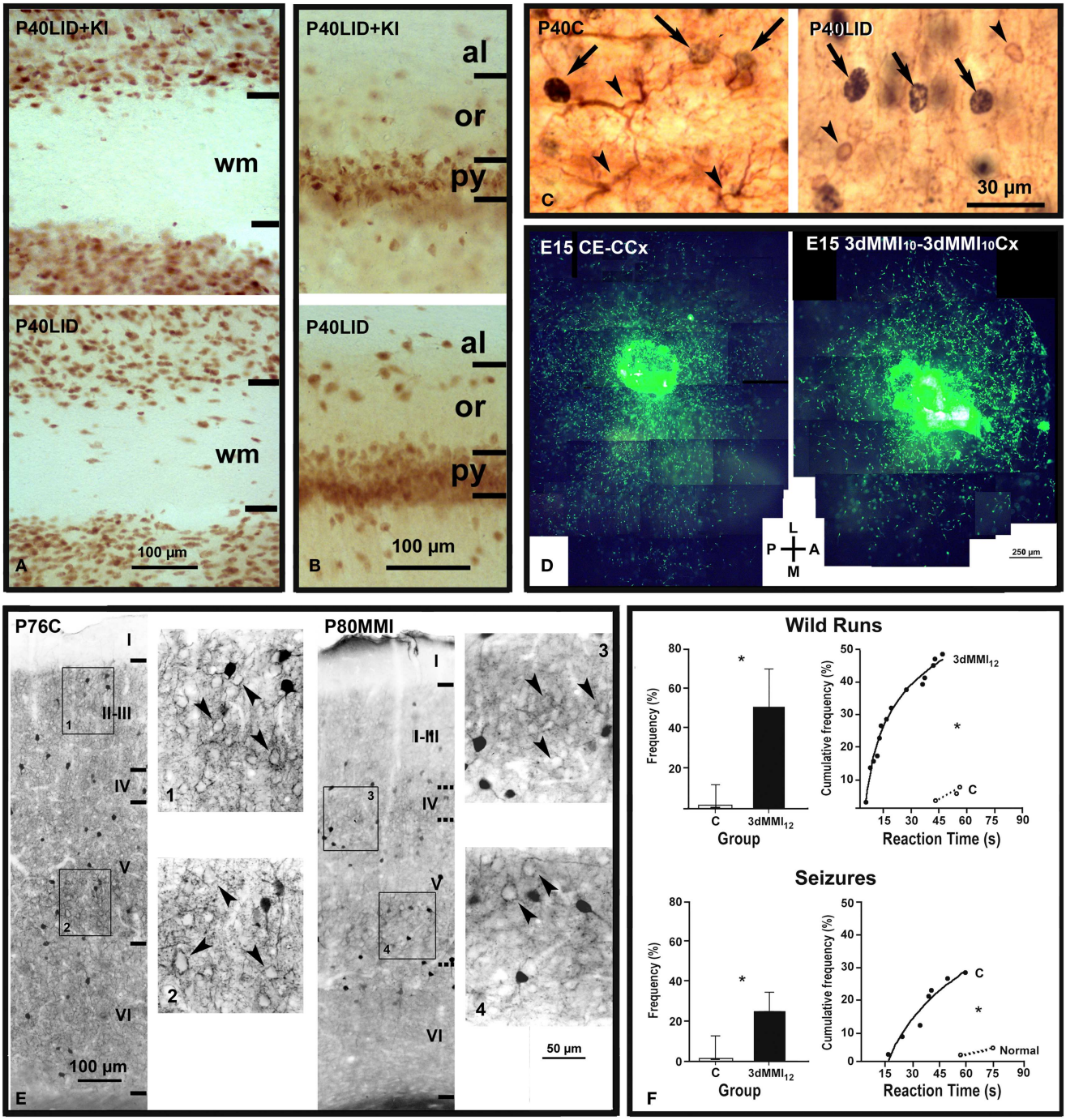

FIGURE 2 | Abnormal neuronal radial and tangential migration, inhibitory local circuits and increased audiogenic seizures in developmental hypothyroidism and hypothyroxinemia. **(A,B)** Photomicrographs of NeuN-immunostained coronal sections of the primary somatosensory cortex **(A)** and hippocampal CA1 **(B)** in LID + KI (rats fed low iodine diet plus approximately 10 µg iodine per day, during gestation and postnatally) and LID (rats fed low iodine diet) progeny at P40. The number of NeuN-labeled neurons increases both in subcortical white matter [wm **(A)**] and in strata oriens (or) and alveus [al **(B)**] of hippocampal CA1 of LID pups as compared with LID + KI pups. **(C)** GFAP- and CNP-positive astrocytes (left panel) and oligodendrocytes (right panel), respectively (arrowheads), and BrdU-positive nuclei (arrows) are shown in layer V of C and LID pups. LID rats received single BrdU injections at E14, E15, and E16. Note that both GFAP-positive astrocytes and CNP-positive oligodendrocytes are BrdU-negative. **(D)** Low power fluorescent photomicrograph collage illustrating the tangential distribution of GFP-MGE control migrating neurons (control explant, CE) in wild control flat cortical mounts at E15 (control cortex, CCx; left), and GFP-MGE hypothyroxinemic migrating neurons ($3dMMI_{10}$) in hypothyroxinemic flat cortical mounts ($3dMMI_{10}Cx$; right). Note that migrating neurons toward the medial (M) region in the hypothyroxinemic cortical mount (right) expand less than those migrating in the control cortical mount (left). $3dMMI_{10}$ rats received MMI treatment

(Continued)

FIGURE 2 | Continued
from E10 to E12. **(E)** Photomicrographs through layer V of the auditory cortex immunostained for parvalbumin in normal (C) and hypothyroid (MMI treatment from E14 onward) rats. In normal rats, immunoreactive cells, processes and perisomatic puncta can be seen. In MMI rats, immunoreactive cells, processes and perisomatic puncta can also be seen but they are less prominent than in normal rats. **(F)** Responses of C and 3dMMI12 (MMI treatment from E12 to E15) pups to an acoustic stimulus. Histograms on the left correspond to the proportion (median with 25th and 75th percentiles) of pups responding with wild runs and with wild runs followed by a seizure, respectively. Graphs on the right represent the cumulative frequency of pups from the same groups that respond with wild runs alone or followed by a seizure, respectively, at the intervals after onset of the stimulus that are shown in the abscissa. (*) Indicates a statistically significant difference compared with control. **(A–C)** Modified from Lavado-Autric et al. (164). **(D)** Modified from Cuevas et al. (159). **(E)** Modified from Berbel et al. (211). **(F)** Modified from Ausó et al. (158).

DELAYED CORTICAL MATURATION

There is a strong evidence that subplate neurons play an important role in thalamocortical axon path finding (233, 234). Subplate neurons may fire action potentials (235) and they are necessary for the establishment of ocular dominance and orientation columns (236) and for the maturation of inhibitory circuits in layer IV (237). The dynamic integration of subplate neurons into the rodent neocortex during postnatal development may play a key role in establishing the cytoarchitectonic pattern in layer IV and to refine layer IV circuitry (238). Recent studies have shown that subplate neurons remain expressing Camk4 in adult hypothyroid rats, while in normal rats, Camk4 is not longer expressed in subplate neurons by P10 [Ref. (239); **Figures 3A–C**]. Subplate and white matter abnormalities have been related to the pathogenesis of various brain developmental disorders other than ASD, such as periventricular leukomalacia, schizophrenia, and cerebral palsy (240–244). A recent study shows the crucial importance of the identification of subplate cell subpopulations, which may have very different roles in various pathologies such as ASD and schizophrenia (245).

Serotonin (5-HT) immunostaining is a good transient marker for thalamic afferents in the visual, auditory, and somatosensory areas of rats during the first postnatal days. In the barrel cortex of hypothyroid rats, immunolabeling persisted for 5 days until P16–17 [Ref. (246); **Figure 3D**]. A similar protracted expression of 5-HT transporter (5-HTT) occurred in the ventro-basal thalamic nucleus and cerebral cortex (246). Reduced 5-HT levels during barrel formation delay the differentiation of layers II, III (247, 248) and reduce the tangential extent of thalamocortical arbors within barrels (249). Thus, prolonged 5-HTT expression in the hypothyroid ventro-basal thalamic nucleus should decrease the concentrations of 5-HT in the extracellular space of sensory cortices, affecting their organization and differentiation.

In the barrel cortex of adult hypothyroid rats, the radial distribution of thalamic afferents, anterogradely labeled with dextran–biotin amine and DiI, was reduced compared to normal rats [Ref. (246); **Figure 3G**]. By single reconstructions of terminal arbors (**Figure 3H**), these authors showed a reduction of the number of axonal branches reaching layers II–IV, and a 49% reduction in the total length of terminal axon arbors in hypothyroid rats. This arrested growth was also reflected by a 58% reduction in the number of buttons per terminal (**Figure 3I**). In hypothyroid rats, ramification of the thalamocortical axons would appear to be stalled postnatally, resulting in reduced synaptogenesis as suggested by the reduced number of buttons in thalamocortical axons [Ref. (246); **Figure 3I**] and in a decreased number of spines along the apical shafts of the hypothyroid pyramidal cells (250). All of the above data show that many target pyramidal cells fail to reach their correct cortical location, not only failing to complete their normal maturation but also that the afferents have arrested growth. In fact, GAP-43 is downregulated, while Sema3A is upregulated in developmentally hypothyroid and hypothyroxinemic pups (251). In agreement, *GAP-43*$^{-/-}$ mice failed to express 5-HTT in the barrel cortex causing a disrupted segregation of thalamic afferents in the barrel cortex. In addition, recent data show that the density of VGluT1-immunoreactive buttons is decreased in layer IV of the parietal cortex of hypothyroid rats (180). These data show that there is an asynchrony in the maturation of thalamocortical afferents and their cortical targets in hypothyroid rats. Cortical cells could be at a stage of maturation that does not allow them to respond to thalamocortical signals, resulting in abnormal communication between thalamic axons and target cells (e.g., by reduced synaptogenesis). Hypothyroidism seems to dissociate stabilization of juvenile axons from maturation, growth in caliber and myelination, processes, which were previously thought to be necessarily linked (134, 232, 252).

Abnormal patterns of connectivity have been also found in the hippocampus of developmentally hypothyroid rats (193). These authors found in the hippocampus of pups born to hypothyroid dams that CA pyramidal neurons developed atrophic apical (15% shorter) and fewer number of ramifications (about 31 and 36% less in dentate gyrus and CA, respectively). Blurred layering and heterotopic neurons were also found in the hippocampus of pups born to hypothyroxinemic pregnant rats [Ref. (158, 164); **Figure 2B**]. Decreased mossy fiber zinc density (33–45% reduction) was found in perinatal hypothyroid rats after PTU treatment from E18 to P31 (253) and in postnatal hypothyroid rats (254). P40 pups born to late hypothyroid dams (thyroidectomized by E16; LMH pups), showed a 41.5% decrease in the Zn-positive area in the stratum oriens, in parallel to down expression of the Zn transporter-3 (ZnT-3; **Figure 3E**) and reduced density of VGluT1-immunoreactive buttons (**Figure 3F**). In addition, pCreb/pATF1, pCreb/Creb, pErk1/Erk2, and pErk2/Erk2 ratios in the hippocampus decreased in LMH pups (59.1, 66.7, 44.4, and 42.9%, respectively) [Ref. (169); **Figures 3J,K**]. Recently, the hippocampus of developmentally hypothyroid pups showed altered VGluT1/VGAT immunoreactivity (180). Although Camk4/Creb pathway plays a fundamental role in neurites growth and establishment of synapses, other genes are involved in the development of hippocampal connections. It has been found that the T3-regulated BDNF is involved in the regulation of the translational expression of VGluT1 in cultured hippocampal neurons (255, 256). Interestingly, BDNF was also found involved in the activation of Erk1/2 signaling pathway (188), which affects not only the differentiation of hippocampal neurons but also almost all

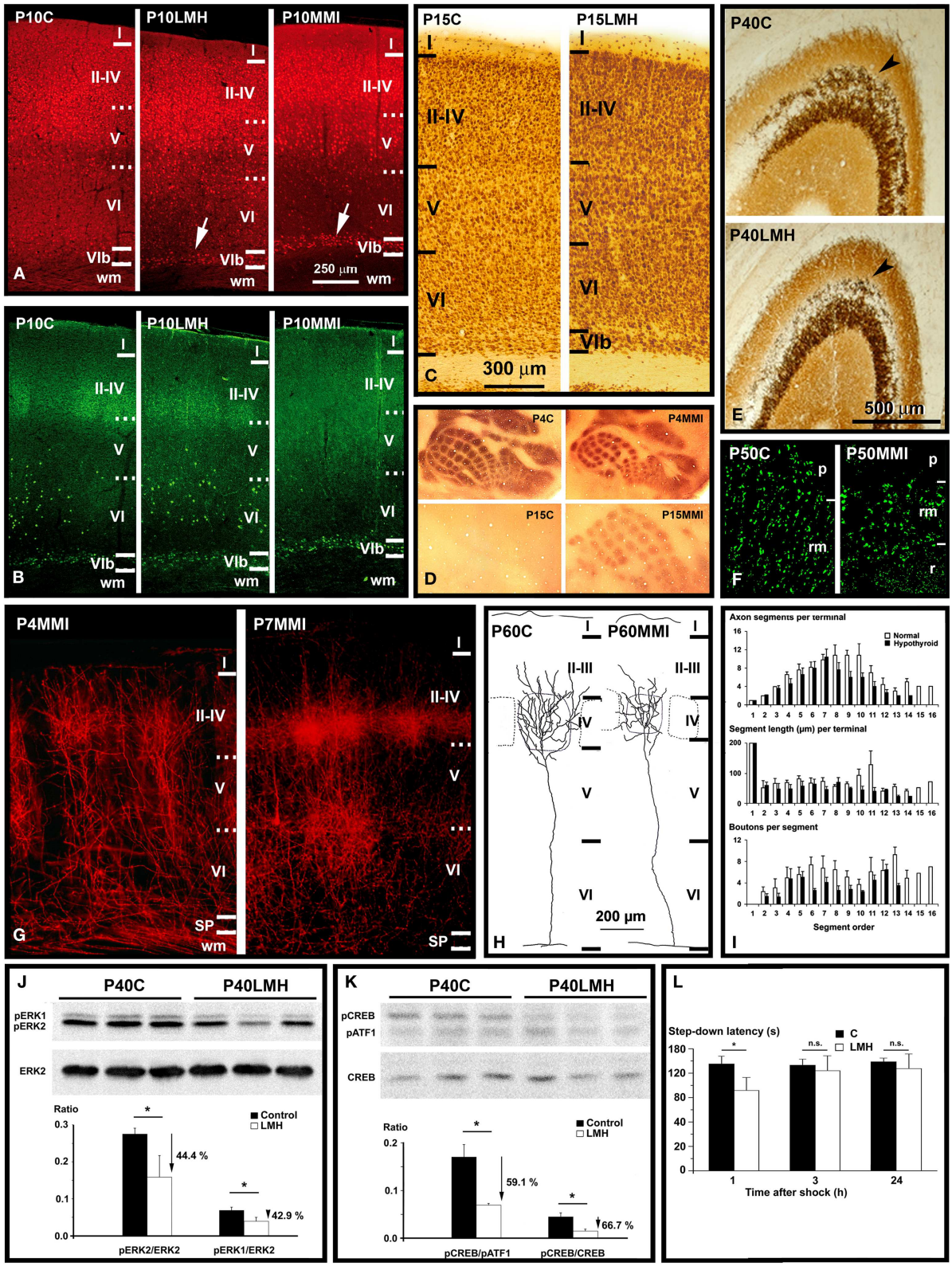

FIGURE 3 | Thyroid hormones affect connectivity, maturation, and function of the cerebral cortex. **(A,B)** Collages from confocal photomicrographs (taken with the ×20 objective) showing double immunolabeling for Camk4 [red; **(A)**] and Nurr1 [green; **(B)**] in the parietal cortex of P10 control (C), LMH (pups born to dams thyroidectomized at E16), and MMI (MMI treatment starting at E10) pups. Most of Camk4-immunoreactive neurons were located in layers II–IV and upper layer V. Numerous

(Continued)

FIGURE 3 | Continued
Camk4-immunoreactive neurons can be seen in layer VIb of P10LMH and P10MMI pups (arrows) compared with P10C pups. At P10, about 60% of Camk4-immunoreactive neurons of layer VIb are also Nurr1-immunoreactive. **(C)** Photomicrographs of coronal sections of the parietal cortex showing NeuN-immunoreactive neurons in C and LMH pups at P15. At P15, the border between the subplate and adjacent layer VI is more clear-cut in LMH than in C pups, showing that a remaining subplate is still present. **(D)** Photomicrographs of flattened neocortex tangential sections showing 5-HT immunostaining in the posteromedial barrel subfield of the parietal cortex of C and MMI rats at P4 and P15. At P4, heavily immunostained barrels can be seen. Decay of 5-HT labeling occurs by P11 in C and by P16 in MMI rats. Note that in P15C rats, no barrels were immunostained, whereas in P15MMI rats, they are still immunopositive. **(E)** Photomicrographs of coronal sections of the area CA3 of the hippocampus showing the Zn-labeling of mossy fibers in C and LMH pups at P40. Note the heavier labeling of mossy fibers in the stratum oriens (arrowhead) of CA3 in C compared with LMH pups. **(F)** Confocal deconvoluted images of vesicular glutamate transporter type 1 (VGluT1, which labels excitatory buttons) of the stratum radiatum (rm) in CA3 in C and MMI rats at P50, labeling mossy fiber buttons. Note the decreased density of immunoreactive buttons in P50MMI pups compared to controls. **(G)** Photomicrographs of coronal sections from P4 and P7 MMI rats that had the lipophilic carbocyanine DiI tracer (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate) implanted in the ventro-basal thalamic nucleus. At P4, DiI-labeled thalamic afferents enter the somatosensory cortex, and form clusters in layer IV. At P7, collaterals in layer IV form more dense clusters than at P4. These images show that hypothyroid thalamic axons reach their somatosensory target areas as in normal rats. **(H)** Coronal views of thalamocortical terminal arbors in layer IV in the posteromedial barrel subfield of C and MMI rats at P60. The barrel limit is marked with dashed lines. Note that in MMI rats terminal arbors have shorter and tortuous branches. **(I)** Histograms representing mean values for the number of axon segments per terminal (upper), segment length (middle), and buttons per segment (bottom) in C (white bars) and MMI (black bars) rats for each segment order. Note that in general MMI mean values are lower. **(J,K)** Western blots obtained from the hippocampus of C and LMH pups at P40, immunolabeled for ERK2, pERK1, and pERK2 **(J)** and pATF1, pCREB, and CREB **(K)**. Histograms showing that the pERK1/ERK2 and pERK2/ERK2 **(J)** and pCREB/pATF1 and pCREB/CREB **(K)** ratios are reduced by 44.4, 42.9, 59.1, and 66.7%, respectively, in LMH compared with C pups. **(L)** Histogram showing step-down latencies in seconds at 1, 3, and 24 h after the initial foot shock in C and LMH pups at P39. Pups from LMH dams show 24.9% reduction in the step-down latency at 1 h after the foot shock. **(J–L)** Error bars represent ± SD; n.s., no significant differences; $*P < 0.001$ for LMH compared with C group. **(A,B)** Modified from Navarro et al. (239). **(C,E,J,K,L)** Modified from Berbel et al. (169). **(D,G,H,I)** Modified from Ausó et al. (246).

aspects of corticogenesis (**Tables 3–6**). However, the inactivation of other pathways such as the Gsk3β/CRMP2 pathway will result in delayed axonal growth (251, 257). Decreased p-Gsk3β labeling and increased labeling of its inactivated p-CRMP2 target protein was seen in the developmentally hypothyroid and hypothyroxinemic rat hippocampus (251).

Altered postnatal synaptogenesis has been observed in the molecular layer of the cerebellar cortex of hypo- and hyperthyroid rats (214). Neurotrophins BDNF and NT3 are downexpressed in postnatal PTU treated rats, resulting in atrophy of Purkinje cell dendrites and in a decreased number of synapses (258) and BDNF is also downexpressed in the hippocampus of developing hypothyroid rats (256). However, neurotrophins might have a dual role in developing and adult hypothyroid rats, because, BDNF is highly expressed in layers II, III, and V of the neocortex and in all hippocampal areas of adult hypothyroid rats (259). These authors observed an increased number of apoptotic neurons and astrocytes in the adult hypothyroid cortex, and they suggested that the increase of BDNF in the hypothyroid adult neocortex and hippocampus might have a protective role against cellular stress associated to degenerative processes. Interestingly, in young and adult autistic human cerebellar samples (ages ranging from 7.5 to 34 years) a 40.3% increase in NT3 expression was found (260). Other T3-regulated genes, involved in synaptogenesis, plasticity, and neurotransmission, have been found [Ref. (23, 44); **Tables 4** and **5**]. The T3-regulated *ANXA6* gene codes for the calcium-binding protein annexin 6 that may play a structural role in facilitating membrane association at the cisternal organelle level and/or in stabilizing IP3R1 microdomains in the axonal initial segment of hippocampal neurons (261). *NR4A1* codes for the transcription factor Nurr77 that mediates in mechanisms of long-term synaptic plasticity in the hippocampus and consequently, in the consolidation of long-term hippocampus-dependent memory (262). *PACSIN2* codes for protein kinase C and casein kinase substrate in neurons protein 2, which interacts with dynamin and synapsin, which affect the recruitment of synaptic vesicles (263), while *TGB2* codes for transforming growth factor-beta 2, which is involved in the differentiation of granule neurons of the dentate gyrus (188).

Structural changes in the cerebral cortex result in altered electrophysiology and behavior of thyroid deficient rats. Most of the electrophysiological studies have been performed in the CA1 hippocampal area. Decreased long-term potentiation (LTP) was shown in developing rats with severe and chronic hypothyroidism (166, 264), as well as in adult hypothyroid rats (265). Altered LTP was also observed in pups born to rats treated with MMI from E12 to E15 (266). Recent studies have shown that developmental hypothyroidism decreases the number of bursting CA1 cells, as well as the number of spikes per burst, resulting from altered low-threshold Ca^{2+} current (267). As mentioned above, the growth of axon caliber was arrested in the anterior commissure and the corpus callosum of hypothyroid rats (225, 228), which might be relevant for the signal transmission velocity of commissural axons (268). The behavior of hypothyroid rats related to cerebral cortex alterations are mainly based on tests to measure (i) locomotor functional excitability and seizure susceptibility (158, 218, 221, 269), and (ii) learning, attention, and memory deficits (169, 219, 264, 266, 270, 271) (**Figure 3L**). Despite the differences between rodent and human cerebral cortices, these studies might be useful to find morphofunctional alterations in hypothyroid rodents that might also occur in neurological and mental disorders associated to hypothyroidism in humans, such as ASD and ADHD. Significant T3-regulated genes, involved in memory and behavior have been found [Ref. (23, 44); **Table 6**]. Among these, *ADCY8*, *ADRBK2*, and *GRK5* code for proteins associated to G-protein signaling (272–274). The transcription factors DBP, involved in kainite-involved seizures and hippocampal plasticity (275), and NR2A1 were mentioned above.

ASD AND THYROID HORMONES DURING BRAIN DEVELOPMENT

Experimental studies in rodents clearly show that thyroid hormone deficiency results in delayed, temporarily, or permanently suppressed, or abnormal, connections, resulting in behavioral and brain dysfunction (10). Abnormal morphophysiological and behavioral traits established during gestation and early postnatal ages might be maintained throughout life and thereby be a risk factor for the development of behavioral and mental disorders later in life.

Despite differences resulting from age at autopsy and the concomitant effects of seizures and of intellectual disability of variable severity, a substantial body of evidence has accumulated since the 1970s on the fundamental morphological changes affecting the brain of patients with ASD (276, 277). Wegiel et al. (278) reviewed available neuropathological data and concluded that ASD results from dysregulation of the normal mechanisms of neurogenesis and neuronal migration, plus dysplastic changes and defects of neuronal maturation. Thus, the neuropathology of ASD is consistent with a prenatal time of onset. A likely etiological hypothesis posits that ASD may be caused by thyroid hormone deficiencies during cerebral cortex development, either due to a genetic deficiency of the *TRIP8* gene (thyroid receptor interacting protein), which codes for a transcriptional regulator associated with nuclear thyroid hormone receptors (279) or associated to maternal hypothyroidism, which increases fourfold the risk of ASD in the child (3, 63).

The morphological brain changes and the genes found to be transcriptionally or functionally involved in ASD will be briefly reviewed here, both from *post-mortem* data and from *in vivo* imaging, along with a summary of the neurotransmitters affected. Finally, the relevance of thyroid hormones in accepted animal models of ASD is presented. Thyroid hormone deficiency diseases and ASD share common altered gene pathways and comorbid disorders, and epidemiological studies reported a relationship between thyroid hormone deficiency and ASD, although morphofunctional differences between these two conditions exist.

BRAIN ALTERATIONS

Young children with ASD are megalencephalic, with increased brain size and weight (276, 277). Brain imaging data and head circumference studies have shown two phases of early brain growth in ASD pathology: early brain overgrowth during the first postnatal years and arrest of growth during early childhood (280), which might be overlapped with neuronal degeneration in some brain regions by preadolescence and continued into adulthood (280, 281). Increased number of neurons could contribute to increase brain volume. Macroscopically and on imaging, the cerebral cortex in ASD exhibits an abnormal pattern of convolutions (282) involving the orbitofrontal cortex (283) and the temporal lobes with hyperconvoluted hippocampus (276). Counts performed in Nissl stained sections suggests increased density of cells in the frontal cortex (284). However, other factors, besides of the increase in the number of neurons, can contribute to increase brain volume. Increased cerebrospinal fluid volume, and slight reductions of gray and white matter volume in frontal, temporal, and parietal lobes have been reported (285). In addition, recent studies have shown focal brain inflammation (286, 287) and increased gliosis subjacent to neuronal degeneration (281). Changes do not affect the brain uniformly, i.e., the fusiform face area and the limbic system have increased cell packing density and smaller neuronal size involving hippocampus, subiculum, and amygdala, and to a lesser extent the entorhinal cortex, mammillary bodies, and septal nuclei (277), while other areas are normal; for instance, the posteroinferior occipitotemporal gyrus showed no differences in pyramidal neuron number or size in layers III, V, and VI (288). In Brodmann areas 44–45, Jacot-Descombes et al. (289) demonstrated reduced pyramidal neuron size suggesting impairment of neuronal networks relevant to communication and social behaviors. However, owing to the relatively small number of autistic brains studied up to date and the enormous heterogeneity in ASD phenotypes and comorbid diseases, more neuropathological studies will be need for clarification of neuroanatomy of ASD (107, 290).

Despite changes in brain volume in ASD, some anatomical alterations are common with hypothyroid brains. Microscopic examination reveals dysgenesis of the cerebral cortex (276, 277) with increased cortical thickness, abnormal laminar patterns, high density of hippocampal neurons, presence of neurons in the molecular layer, neuronal disorganization, poor differentiation of the gray–white matter boundary, and neuronal heterotopias. Cortical neurons are small, closely packed, lack dendritic arbors, and appear immature; these changes are consistent with an arrest of cerebral maturation (290, 291). Also, the cortical organization is altered with narrower cortical minicolumns (292, 293). The focal cortical dysplasia of ASD appears to result from loss of synchronized radial and tangential migration of glutamatergic and GABAergic neurons, respectively (294). The *CNTNAP2* gene, which codes for contactin associated protein-like 2, is expressed in human frontal areas and has been found to be involved in ASD and language impairment (295, 296). A finding consistent with this view is the demonstration by Kotagiri et al. (297) of cytoarchitectural changes in the ependymal cells of the subventricular zone in ASD, with lower cell density in the septal but not in the striatal zone. A subset of ependymal, astrocyte ribbon, and rostral migratory stream (RMS) cells expressed PCNA, Ki67, PLP, and α-tubulin. In addition, the white matter shows areas of focal increase in the number of heterotopias, reflecting abnormal neuronal migration (278). Using imaging, Gozzi et al. (298) showed that the magnetization transfer ratio of the corpus callosum was significantly higher in children with ASD than in normal controls, indicating abnormal myelination in ASD.

According to a consensus by Fatemi et al. (299), reduction in Purkinje cell and cerebellar granule cell density is consistently observed in ASD (300), along with developmental abnormalities of the inferior olives (301), consistent with abnormal neuronal migration before the 3rd month of gestation. Purkinje cells are decreased in the posterolateral neocerebellar cortex and the archicerebellar cortex (302) with vermis hypoplasia on brain imaging (303, 304). Using MRI tractography in children with ASD, Jeong et al. (305) showed decreased fiber numbers connecting cerebellar cortex to ventral and dorsal dentate nuclei confirming a decrease in connectivity and numbers of Purkinje cells.

NEUROTRANSMITTERS IN ASD

Perry et al. (306) investigated cholinergic biomarkers in the basal forebrain, frontal cortex, and parietal cortex of children with ASD, mental retardation, and epilepsy and found decreased binding of the α4 nicotinic and the muscarinic M1 receptors (α4 nAChR and m1AChR, respectively). In the cerebellum, Lee et al. (307) found decreased α3 and α4 nAChR binding in granule cells, Purkinje cells, and molecular layers along with increased α7 nAChR binding in the granule cell layer. Blatt et al. (308) found that only the GABAergic system was significantly reduced in the hippocampus in ASD; the serotoninergic, cholinergic, and glutamatergic systems were normal. $GABA_A$ and $GABA_B$ receptor density in the anterior cingulate cortex and fusiform gyrus is decreased (309, 310). The dysregulation of the GABAergic system pathway includes downregulation of $GABA_A$ and $GABA_B$ receptors (309–311) and reduction of glutamic acid decarboxylase enzymes (312) and metabotropic glutamate receptor type 5 [mGluR5; (313)]. FMRP and mGluR5 are reduced in cerebellar vermis and frontal cortex in ASD (314, 315). In addition, 5-HT neurotransmission has been found to be deficient in ASD; in particular, Oblak et al. (316) showed decrease in 5-HT_{1A} receptor and 5-HT_{2A} receptor-binding density, as well as in 5-HTT in posterior cingulate cortex and fusiform gyrus. Mutations in the $GABA_A$ receptor subunit have been associated with ASD and epilepsy (317).

Two relevant genes in the diagnosis of ASD are *SHANK3* (318) and *GABRB3* (311). SHANK3 is a synaptic scaffolding protein enriched in the postsynaptic density of excitatory synapses, and plays important roles in the formation, maturation, and maintenance of synapses. Several *SHANK3* mutations have been identified in a particular phenotypic group of patients with ASD (318). A study of the Danish Newborn Screening Biobank revealed levels of BDNF in the lower 10th percentile during the neonatal period in children later diagnosed with ASD (319). *SHANK3* mutations may be involved in ASD, cerebellar development, and cerebellar vermis hypoplasia (320). *GABRB3* codes for $GABA_A\beta 3$ receptor, and is downexpressed in brains of autistic children, particularly in the cerebellum (311).

THYROID-RELATED GENES INVOLVED IN ASD

Recently, Betancur (321) concluded that despite the more than 100 genetic and genomic disorders associated with ASD, we still lack a clear understanding of its pathogenesis. In 2007, Castermans et al. (279) identified in a subject with ASD a *de novo* chromosomal anomaly on chromosome 10q21.3 that disrupted the *TRIP8* gene and the nearby *REEP3* gene that codes for receptor expression-enhancing protein 3, which is a microtubule associated protein sequestering the endoplasmic reticulum away from chromosomes during mitosis. The authors concluded that *TRIP8* codes for a protein predicted to be a transcriptional regulator associated with nuclear thyroid hormone receptors but noted that, "no link between thyroid gland and ASD has been reported so far."

We summarize in **Tables 1–6** a list of relevant genes that have been found to be T3-regulated at the transcriptional level in the rodents cerebral cortex (149, 322), and their human homolog genes (marked in bold) that have been found mutated in ASD patients. The list is far to be exhaustive and, most probably, the overlapping between T3-regulated and ASD-mutated (T3/ASD) genes will increase in the near future. Relevant are Creb/Crem transcription factors that are involved in all critical events of corticogenesis, and Camk4 and Erk1/2 kinases that participate in the Camk4/Creb/Crem and Erk1/2/Creb/Crem signaling pathways (44, 183, 323–325).

As mentioned earlier, critical events at the beginning of corticogenesis are cell division and differentiation of neuroblasts to become young migrating neurons, and the migration of young neurons to their final destinations. T3/ASD genes involved in cell division and differentiation (**Table 1**) are *CTNNB1* codes for β-catenin that is involved in the transition of epithelial-to-mesenchymal transition (symmetrical-to-asymmetrical divisions; see above) and in the astrocytes' differentiation (326); *DYRK1A* codes for dual specificity tyrosine-phosphorylation-regulated kinase 1A, which is a regulator of brain growth (196); *GNB1L* code for a 6 WD40 repeats-containing protein most likely involved in cell cycle regulation (327); and *FLT1* that codes for vascular endothelial growth factor receptor 1, a tyrosine kinase involved in the control of cell proliferation and differentiation in angiogenesis and neurogenesis; *FLT1* has been found reduced in severe autism (328). T3/ASD genes involved in cytoskeleton organization and cell migration (**Table 2**) are *GNAS* that codes for G-protein α subunit (Gs-α) (329); *FN1* that codes for fibronectin, an extracellular matrix protein involved in cell adhesion and migration, found increased in serum of children with autism (330); *SERPINH1* that codes for heat shock protein 47 that binds collagen and was found abnormally expressed in the temporal cortex of ASD patients (331); and *NEFH*, *NEFM*, and *NEFL* code for neurofilament subunits and has been found altered in the frontal cortex neurons in children with autism (332). The genes involved in the reelin signaling pathway include *RELN* (reelin), *DAB1* (disabled-1), *VLDLR* (very-low-density-lipoprotein receptor) (331–335), and *PAFAH1B1* (platelet-activating factor acetylhydrolase IB subunit α; Lis1) that interacts with dimein and VLDLR. Fatemi et al. (312) reported decreased blood levels of reelin in children with ASD. Also, using post-mortem material from superior frontal, parietal, and cerebellar cortex from autistic brains and matched controls, significant reductions in reelin protein, reelin mRNA, and dab1 mRNA along with elevations in VLDLR mRNA in frontal and cerebellar cortex, indicative of impairments in the reelin/dab1 signaling pathway in ASD were observed (336). Genetic susceptibility polymorphisms of the *RELN* gene have been described in ASD (337–340), although other studies have been negative (341–344). A recent meta-analysis by Wang et al. (345) revealed that the RELN variant rs362691, rather than rs736707 or the GGC repeat variant, might contribute significantly to ASD risk.

The T3/ASD genes involved in neurite development and maturation (**Table 3**) are *ANK3* that codes for ankyrin-3, which participates in the recruitment of voltage-gated sodium channels at the axon hillock and node of Ranvier (346); *ARX* that codes for the transcription factor Aristaless-Related Homeobox, associated to several neurological and psychiatric disorders, including ASD (347, 348); BDNF has high-affinity for TrkB receptor and is involved in neurite development, neuronal plasticity, LTP, and apoptosis of CNS neurons (348, 349); *CNTN4* codes for contactin-4, an Ig-cell adhesion molecule involved in the development

and plasticity of neuronal circuits (350); NOS1 codes for nitric oxide synthase 1 that is involved in glutamate-mediated neurotransmission and toxicity (351); *FLT1*, *FN1*, and *NEFs* were mentioned above. T3/ASD genes involved in synaptogenesis and plasticity (**Table 4**) are *ATP2B2* that codes for plasma membrane calcium-ATPase, involved in the translocation of calcium to the endoplasmic reticulum (352); *NRGN* that codes for neurogranin, involved in synaptic plasticity and LTP (353); *BDNF*, *CNTN4*, and *PAFAH1B1* mentioned above.

The T3/ASD genes involved in neurotransmission (**Table 5**) are *HOMER1* that codes for homer protein homolog 1, is a major component of postsynaptic density involved in metabotropic glutamate receptor signaling (354); *KCNJ10* that codes for ATP-sensitive inward rectifier potassium channel 10, involved in axonal membrane repolarization (355); *NTS* that codes for neurotensin is involved in modulation of dopamine signaling and focal brain inflammation, and was found increased in serum of ASD children (286); *SLC17A7* codes for vesicular glutamate transporter 1 (VGluT1), and is involved in glutamatergic transmission (333); *NRGN* and *PAFAH1B1* were mentioned above.

The T3/ASD genes involved in memory and behavior (**Table 6**) are *CALB1* and *PVALB* that encode calbindin-D28k and parvalbumin, respectively, are involved in GABAergic transmission (332); *HTR7* that codes 5-HT7 receptor is involved in serotonin signal transduction (333, 356); *HOMER1*, *NOS1*, and *NTS* were mentioned above.

ANIMAL MODELS OF ASD

A number of animal models of ASD are the result of insertion/deletion of different ASD-related genes and exposure to environmental factors [reviewed by Gadad et al. and Provenzano et al. (357, 358)]. Sadamatsu et al. (359) proposed the rat with mild and transient neonatal hypothyroidism as a novel model for ASD. Other models include the repetitive behavior observed in C58/J, C57BL/6J, and Grin1 knockdown mice (360). The homeobox-containing transcription factor engrailed-2 (En2) is involved in patterning and neuronal differentiation; Sgadò et al. (361, 362) showed that adult $En2^{-/-}$ mice exhibit reduced brain interneuron expression of GABAergic marker mRNAs, and reduction in parvalbumin, somatostatin, and neuropeptide Y in the cerebellum and cerebral cortex (including hippocampus). The genetically inbred *BTBR* $T^{+}Itpr3^{tf/J}$ mouse model of ASD exhibits social impairment and stereotypic behavior suggestive of mTOR overactivation (363). The BTBR model shows extensive anatomical abnormalities in the white matter of the corpus callosum and the hippocampal commissure (364). Uchino and Waga (365) identified novel *SHANK3* transcripts whose transcription started at the vicinity of the CpG-island 2 in the mouse brain and developed the Shank3 mutant mice that exhibit autistic-like behaviors. Waga et al. (366) identified two different amino-terminus truncated Shank3 transcripts, Shank3c-3 and Shank3c-4, expressed from the intron 10 of the Shank3 gene, and suggested the epigenetic regulation of the expression of these transcripts via methyl CpG-binding protein 2 (MeCP2). Interestingly, MeCP2 mediates activity-dependent regulation of synaptic strength during the process of circuit formation and prevents uncontrolled recurrent excitation that may result in a pathophysiological increase of neuronal excitability, aberrant network activity, and seizures, which are common Rett patients (182).

The valproic acid model of ASD has become widely used (367–371). However, it is not widely known that valproic acid at the usual therapeutic doses used for the treatment of epilepsy has anti-thyroid effects (372) and induces hearing loss in patients (373).

CONCLUSION

Thyroid hormones exert both genomic and non-genomic actions in many tissues, organs, and systems over the course of a lifetime. In particular, they are crucial during early neurodevelopment, since key phases of the CNS development depend of the expression of thyroid hormones regulated genes. These genes affect, among other things, proliferation, migration, and maturation of neurons and glial cells, which under certain circumstances can result in abnormal connectivity, and consequently in behavioral dysfunction. Morphofunctional alterations caused during pregnancy and early postnatal are permanent, and thus they are a risk factor for the development of behavioral and mental disorders later in life. The knowledge of how thyroid hormones regulate these phases of development may help to understand altered regulatory mechanisms in neurodevelopmental diseases such as ASD, ADHD, schizophrenia, and epilepsy with cytoarchitectonic alterations similar to those found in hypothyroidism and hypothyroxinemia and vice versa. By combining basic and clinical investigation, new data will be obtained to better understand the basic phases of brain development and the genetic and physiological events underlying some of the human diseases mentioned above. Despite of obvious differences between humans and other mammals in cortical organization and function, animal models might be a useful tool to approach the understanding of common etiological factors in hypothyroidism and ASD since, as the evo-devo tell us, both rodents and humans share homologous gene pathways involved in these diseases.

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REFERENCES

1. De Robertis EM. Evo-devo: variations on ancestral themes. *Cell* (2008) **132**:185–95. doi:10.1016/j.cell.2008.01.003
2. Rakic P. Evolution of the neocortex: a perspective from developmental biology. *Nat Rev Neurosci* (2009) **10**:724–35. doi:10.1038/nrn2719
3. Román GC, Ghassabian A, Bongers-Shokking JJ, Jaddoe VW, Hofman A, de Rijke YB, et al. Association of gestational maternal hypothyroxinemia and increased autism risk. *Ann Neurol* (2013) **74**:733–42. doi:10.1002/ana.23976
4. Stiles J. Brain development and the nature versus nurture debate. *Prog Brain Res* (2011) **189**:3–22. doi:10.1016/B978-0-444-53884-0.00015-4
5. Román GC. Nutritional disorders in tropical neurology. *Handb Clin Neurol* (2013) **114**:381–404. doi:10.1016/B978-0-444-53490-3.00030-3
6. Legrand J. Hormones thyroïdiennes et maturation du système nerveux. *J Physiol (Paris)* (1983) **78**:603–52.
7. Morreale de Escobar G, Escobar del Rey F. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *N Engl J Med* (1999) **341**:2015–6. doi:10.1056/NEJM199912233412613

8. Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, et al. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *N Eng Med J* (1999) **341**:549–95. doi:10.1056/NEJM199908193410801
9. Zoeller RT, Rovet J. Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. *J Neuroendocrinol* (2004) **16**:809–18. doi:10.1111/j.1365-2826.2004.01243.x
10. Berbel P, Obregón MJ, Bernal J, Escobar del Rey F, Morreale de Escobar G. Iodine supplementation during pregnancy: a public health challenge. *Trends Endocrinol Metab* (2007) **18**:338–43. doi:10.1016/j.tem.2007.08.009
11. Berbel P, Bernal J. Hypothyroxinemia: a subclinical condition affecting neurodevelopment. *Expert Rev Endocrinol Metab* (2010) **5**:563–75. doi:10.1586/eem.10.37
12. Ahmed OM, El-Gareib AW, El-Bakry AM, Abd El-Tawab SM, Ahmed RG. Thyroid hormones states and brain development interactions. *Int J Dev Neurosci* (2008) **26**:147–209. doi:10.1016/j.ijdevneu.2007.09.011
13. Morreale de Escobar G, Ares S, Berbel P, Obregón MJ, Escobar del Rey F. The changing role of maternal thyroid hormone in fetal brain development. *Semin Perinatol* (2008) **32**:380–6. doi:10.1053/j.semperi.2008.09.002
14. Williams GR. Neurodevelopmental and neurophysiological actions of thyroid hormone. *J Neuroendocrinol* (2008) **20**:784–94. doi:10.1111/j.1365-2826.2008.01733.x
15. Stagnaro-Green A, Pearce E. Thyroid disorders in pregnancy. *Nat Rev Endocrinol* (2012) **8**:650–8. doi:10.1038/nrendo.2012.171
16. Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. *Endocr Rev* (2010) **31**:139–70. doi:10.1210/er.2009-0007
17. Aranda A, Pascual A. Nuclear hormone receptors and gene expression. *Physiol Rev* (2001) **81**:1269–304.
18. Forrest D, Reh TA, Rüsch A. Neurodevelopmental control by thyroid hormone receptors. *Curr Opin Neurobiol* (2002) **12**:49–56. doi:10.1016/S0959-4388(02)00289-1
19. Anderson GW, Schoonover CM, Jones SA. Control of thyroid hormone action in the developing rat brain. *Thyroid* (2003) **13**:1039–56. doi:10.1089/105072503770867219
20. Bernal J. Thyroid hormones and brain development. *Vitam Horm* (2005) **71**:95–122. doi:10.1016/S0083-6729(05)71004-9
21. Flamant F, Gauthier K, Samarut J. Thyroid hormones signaling is getting more complex: STORMs are coming. *Mol Endocrinol* (2007) **21**:321–33. doi:10.1210/me.2006-0035
22. Bernal J, Morte B. Thyroid hormone receptor activity in the absence of ligand: physiological and developmental implications. *Biochem Biophys Acta* (2013) **1830**:3893–9. doi:10.1016/j.bbagen.2012.04.014
23. Chatonnet F, Guyot R, Benoît G, Flamant F. Genome-wide analysis of thyroid hormone receptors shared and specific functions in neural cells. *Proc Natl Acad Sci U S A* (2013) **110**:E766–75. doi:10.1073/pnas.1210626110
24. Dumitrescu AM, Refetoff S. The syndromes of reduced sensitivity to thyroid hormone. *Biochim Biophys Acta* (2013) **1830**:3987–4003. doi:10.1016/j.bbagen.2012.08.005
25. Refetoff S, Bassett JH, Beck-Peccoz P, Bernal J, Brent G, Chatterjee K, et al. Classification and proposed nomenclature for inherited defects of thyroid hormone action, cell transport, and metabolism. *Thyroid* (2014) **24**:407–9. doi:10.1089/thy.2013.3393.nomen
26. Friesema EC, Jansen J, Milici C, Visser TJ. Thyroid hormone transporters. *Vitam Horm* (2005) **70**:137–67. doi:10.1016/S0083-6729(05)70005-4
27. Abe T, Suzuki T, Unno M, Tokui T, Ito S. Thyroid hormone transporters: recent advances. *Trends Endocrinol Metab* (2002) **13**:215–20. doi:10.1016/S1043-2760(02)00599-4
28. Sugiyama D, Kusuhara H, Taniguchi H, Ishikawa S, Nozaki Y, Aburatani H, et al. Functional characterization of rat brain-specific organic anion transporter (Oatp14) at the blood-brain barrier: high affinity transporter for thyroxine. *J Biol Chem* (2003) **278**:43489–95. doi:10.1074/jbc.M306933200
29. Bernal J. Role of monocarboxylate anion transporter 8 (MCT8) in thyroid hormone transport: answers from mice. *Endocrinology* (2006) **147**:4034–5. doi:10.1210/en.2006-0695
30. Braun D, Kinne A, Bräuer AU, Sapin R, Klein MO, Köhrle J, et al. Developmental and cell type-specific expression of thyroid hormone transporters in the mouse brain and in primary brain cells. *Glia* (2011) **59**:463–71. doi:10.1002/glia.21116
31. Rodrigues TB, Ceballos A, Grijota-Martínez C, Nuñez B, Refetoff S, Cerdán S, et al. Increased oxidative metabolism and neurotransmitter cycling in the brain of mice lacking the thyroid hormone transporter SLC16A2 (MCT8). *PLoS One* (2013) **8**(10):e74621. doi:10.1371/journal.pone.0074621
32. Müller J, Heuer H. Expression pattern of thyroid hormone transporters in the postnatal mouse brain. *Front Endocrinol* (2014) **5**:92. doi:10.3389/fendo.2014.00092
33. Wirth EK, Schweizer U, Köhrle J. Transport of thyroid hormone in brain. *Front Endocrinol* (2014) **5**:98. doi:10.3389/fendo.2014.00098
34. Guadaño-Ferraz A, Escámez MJ, Morte B, Vargiu P, Bernal J. Transcriptional induction of RC3/neurogranin by thyroid hormone: differential neuronal sensitivity is not correlated with thyroid hormone receptor distribution in the brain. *Brain Res Mol Brain Res* (1997) **49**:37–44. doi:10.1016/S0169-328X(97)00119-8
35. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine-selenodeiodinases. *Endocr Rev* (2002) **23**:38–89. doi:10.1210/edrv.23.1.0455
36. Bianco AC, Kim BW. Deiodinases: implications of the local control of thyroid hormone action. *J Clin Invest* (2006) **116**:2571–9. doi:10.1172/JCI29812
37. Morte B, Bernal J. Thyroid hormone action: astrocyte-neuron communication. *Front Endocrinol* (2014) **5**:82. doi:10.3389/fendo.2014.00082
38. Visser TJ, van Haasteren GA, Linkels E, Kaptein E, van Toor H, de Greef WJ. Gender-specific changes in thyroid hormone-glucuronidating enzymes in rat liver during short-term fasting and long-term food restriction. *Eur J Endocrinol* (1996) **135**:489–97. doi:10.1530/eje.0.1350489
39. Flamant F, Baxter JD, Forrest D, Refetoff S, Samuels H, Scanlan TS, et al. International union of pharmacology. LIX. The pharmacology and classification of the nuclear receptor superfamily: thyroid hormone receptors. *Pharmacol Rev* (2006) **58**:705–11. doi:10.1124/pr.58.4.3
40. Oetting A, Yen PM. New insights into thyroid hormone action. *Best Pract Res Clin Endocrinol Metab* (2007) **21**:193–208. doi:10.1016/j.beem.2007.04.004
41. Bernal J, Pekonen F. Ontogenesis of the nuclear 3,5,3'-triiodothyronine receptor in the human fetal brain. *Endocrinology* (1984) **114**:677–9. doi:10.1210/endo-114-2-677
42. Perez-Castillo A, Bernal J, Ferreiro B, Pans T. The early ontogenesis of thyroid hormone receptor in the rat fetus. *Endocrinology* (1985) **117**:2457–61. doi:10.1210/endo-117-6-2457
43. Bradley DJ, Towle HC, Young WS III. Spatial and temporal expression of alpha- and beta-thyroid hormone receptor mRNAs, including the beta 2-subtype, in the developing mammalian nervous system. *J Neurosci* (1992) **12**:2288–302.
44. Morte B, Díez D, Ausó E, Belinchón MM, Gil-Ibáñez P, Grijota-Martínez C, et al. Thyroid hormone regulation of gene expression in the developing rat fetal cerebral cortex: prominent role of the Ca2+/calmodulin-dependent protein kinase pathway. *Endocrinology* (2010) **151**:810–20. doi:10.1210/en.2009-0958
45. Casas F, Rochard P, Rodier A, Cassar-Malek I, Marchal-Victorion S, Wiesner RJ, et al. A variant form of the nuclear triiodothyronine receptor c-ErbAalpha1 plays a direct role in regulation of mitochondrial RNA synthesis. *Mol Cell Biol* (1999) **19**:7913–24.
46. Casas F, Pessemesse L, Grandemange S, Seyer P, Baris O, Gueguen N, et al. Overexpression of the mitochondrial T3 receptor induces skeletal muscle atrophy during aging. *PLoS One* (2009) **4**(5):e5631. doi:10.1371/journal.pone.0005631
47. Wang D, Xia X, Liu Y, Oetting A, Walker RL, Zhu Y, et al. Negative regulation of TSHalpha target gene by thyroid hormone involves histone acetylation and corepressor complex dissociation. *Mol Endocrinol* (2009) **23**:600–9. doi:10.1210/me.2008-0389
48. Xu M, Iwasaki T, Shimokawa N, Sajdel-Sulkowska EM, Koibuchi N. The effect of low dose lipopolysaccharide on thyroid hormone-regulated actin cytoskeleton modulation and type 2 iodothyronine deiodinase activity in astrocytes. *Endocr J* (2013) **60**:1221–30. doi:10.1507/endocrj.EJ13-0294
49. 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**:889–99. doi:10.1172/JCI114790
50. Kester MH, Martínez de Mena R, Obregón MJ, Marinkovic D, Howatson A, Visser TJ, et al. Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. *J Clin Endocrinol Metab* (2004) **89**:3117–28. doi:10.1210/jc.2003-031832
51. Huang CB, Chen FS, Chung MY. Transient hypothyroxinemia of prematurity is associated with abnormal cranial ultrasound and illness severity. *Am J Perinatol* (2002) **19**:139–47. doi:10.1055/s-2002-25308

52. Chan SY, Vasilopoulou E, Kilby MD. The role of the placenta in thyroid hormone delivery to the fetus. *Nat Clin Pract Endocrinol Metab* (2009) **5**:45–54. doi:10.1038/ncpendmet1026
53. Glinoer D. The importance of iodine nutrition during pregnancy. *Public Health Nutr* (2007) **10**:1542–6. doi:10.1017/S1368980007360886
54. Gaitan JE, Mayoral LG, Gaitan E. Defective thyroidal iodine concentration in protein-calorie malnutrition. *J Clin Endocrinol Metab* (1983) **57**:327–33. doi:10.1210/jcem-57-2-327
55. Vanderpas J. Nutritional epidemiology and thyroid hormone metabolism. *Annu Rev Nutr* (2006) **26**:293–322. doi:10.1146/annurev.nutr.26.010506.103810
56. Leung AM, Pearce EN, Braverman LE. Iodine nutrition in pregnancy and lactation. *Endocrinol Metab Clin North Am* (2011) **40**:765–77. doi:10.1016/j.ecl.2011.08.001
57. Berbel P, Morreale de Escobar G. Iodine and brain development. In: Preedy VR, Watson RR, Martin CR, editors. *International Handbook of Behavior, Food and Nutrition.* New York, NY: Springer Press (2011). p. 2105–34.
58. Köhrle J. Selenium and the thyroid. *Curr Opin Endocrinol Diabetes Obes* (2013) **20**:441–8. doi:10.1097/01.med.0000433066.24541.88
59. Howdeshell KL. A model of the development of the brain as a construct of the thyroid system. *Environ Health Perspect* (2002) **110**:337–48. doi:10.1289/ehp.02110s3337
60. Zoeller TR, Dowling AL, Herzig CT, Iannacone EA, Gauger KJ, Bansal R. Thyroid hormone, brain development, and the environment. *Environ Health Perspect* (2002) **110**:355–61. doi:10.1289/ehp.02110s3355
61. Koibuchi N, Iwasaki T. Regulation of brain development by thyroid hormone and its modulation by environmental chemicals. *Endocr J* (2006) **53**:295–303. doi:10.1507/endocrj.KR-69
62. Kimura-Kuroda J, Nagata I, Kuroda Y. Disrupting effects of hydroxy-polychlorinated biphenyl (PCB) congeners on neuronal development of cerebellar Purkinje cells: a possible causal factor for developmental brain disorders? *Chemosphere* (2007) **67**:S412–20. doi:10.1016/j.chemosphere.2006.05.137
63. Román GC. Autism: transient in utero hypothyroxinemia related to maternal flavonoid ingestion during pregnancy and to other environmental antithyroid agents. *J Neurol Sci* (2007) **262**:15–26. doi:10.1016/j.jns.2007.06.023
64. Parent AS, Naveau E, Gerard A, Bourguignon JP, Westbrook GL. Early developmental actions of endocrine disruptors on the hypothalamus, hippocampus, and cerebral cortex. *J Toxicol Environ Health B Crit Rev* (2011) **14**:328–45. doi:10.1080/10937404.2011.578556
65. Gilbert ME, Rovet J, Chen Z, Koibuchi N. Developmental thyroid hormone disruption: prevalence, environmental contaminants and neurodevelopmental consequences. *Neurotoxicology* (2012) **33**:842–52. doi:10.1016/j.neuro.2011.11.005
66. de Cock M, Maas YG, van de Bor M. Does perinatal exposure to endocrine disruptors induce autism spectrum and attention deficit hyperactivity disorders? *Acta Paediatr* (2012) **101**:811–8. doi:10.1111/j.1651-2227.2012.02693.x
67. Zoeller RT, Brown TR, Doan LL, Gore AC, Skakkebaek NE, Soto AM, et al. Endocrine-disrupting chemicals and public health protection: a statement of principles from the endocrine society. *Endocrinology* (2012) **153**:4097–110. doi:10.1210/en.2012-1422
68. Bizhanova A, Kopp P. Controversies concerning the role of pendrin as an apical iodide transporter in thyroid follicular cells. *Cell Physiol Biochem* (2009) **28**:485–90. doi:10.1159/000335103
69. Langer P. The impacts of organochlorines and other persistent pollutants on thyroid and metabolic health. *Front Neuroendocrinol* (2010) **31**:497–518. doi:10.1016/j.yfrne.2010.08.001
70. Iavicoli I, Fontana L, Leso V, Bergamaschi A. The effects of nanomaterials as endocrine disruptors. *Int J Mol Sci* (2013) **14**:16732–801. doi:10.3390/ijms140816732
71. Hinther A, Vawda S, Skirrow RC, Veldhoen N, Collins P, Cullen JT, et al. Nanometals induce stress and alter thyroid hormone action in Amphibia at or below North American water quality guidelines. *Environ Sci Technol* (2010) **44**:8314–21. doi:10.1021/es101902n
72. Hetzel BS. Iodine deficiency disorders (IDD) and their eradication. *Lancet* (1983) **2**:1126–9. doi:10.1016/S0140-6736(83)90636-0
73. Delange F. The disorders induced by iodine deficiency. *Thyroid* (1994) **4**:107–28. doi:10.1089/thy.1994.4.107
74. Morreale de Escobar G, Obregón MJ, Escobar del Rey F. Maternal thyroid hormones early in pregnancy and fetal brain development. *Best Pract Res Clin Endocrinol Metab* (2004) **18**:225–48. doi:10.1016/j.beem.2004.03.012
75. Glinoer D. The regulation of thyroid function during normal pregnancy: importance of the iodine nutrition status. *Best Pract Res Clin Endocrinol Metab* (2004) **18**:133–52. doi:10.1016/j.beem.2004.03.001
76. Dumont JE, Optiz R, Christophe D, Vassart G, Roger PP, Maenhaut C. The phylogeny, ontogeny, anatomy and regulation of the iodine metabolizing thyroid. In: DeGroot LJ, editor. *Thyroid Disease Manager*. South Dartmouth, MA: Endocrine Education, Inc (2008). p. 1–109.
77. Leung AM, Pearce EN, Braverman LE. Perchlorate, iodine and the thyroid. *Best Pract Res Clin Endocrinol Metab* (2010) **24**:133–41. doi:10.1016/j.beem.2009.08.009
78. Azizi F, Smyth P. Breast feeding and maternal and infant iodine nutrition. *Clin Endocrinol (Oxf)* (2009) **70**:803–9. doi:10.1111/j.1365-2265.2008.03442.x
79. Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, et al. Guidelines of the American thyroid association for the diagnosis and management of thyroid disease during pregnancy and postpartum. *Thyroid* (2011) **21**:1081–125. doi:10.1089/thy.2011.0087
80. Azizi F, Aminorroya A, Hedayati M, Rezvanian H, Amini M, Mirmiran P. Urinary iodine excretion in pregnant women residing in areas with adequate iodine intake. *Public Health Nutr* (2003) **6**:95–8. doi:10.1079/PHN2002366
81. Travers CA, Guttikonda K, Norton CA, Lewis PR, Mollart LJ, Wiley V, et al. Iodine status in pregnant women and their newborns: are our babies at risk of iodine deficiency? *Med J Aust* (2006) **184**:617–20.
82. Lazarus JH, Smyth PPA. Iodine deficiency in the UK and Ireland. *Lancet* (2008) **372**:888. doi:10.1016/S0140-6736(08)61390-2
83. Andersson M, Karumbunathan V, Zimmermann MB. Global iodine status in 2011 and trends over the past decade. *J Nutr* (2012) **142**:744–50. doi:10.3945/jn.111.149393
84. Leung AM, Pearce EN, Braverman LE. Sufficient iodine intake during pregnancy: just do it. *Thyroid* (2013) **23**:7–8. doi:10.1089/thy.2012.0491
85. World Health Organization. *Trace Elements in Human Nutrition and Health.* Geneva: World Health Organization (1996).
86. Hetzel BS. Iodine and neuropsychological development. *J Nutr* (2000) **130**:493S–5S.
87. Pop VJ, Brouwers EP, Vader HL, Vulsma T, van Baar AL, de Vijlder JJ. Maternal hypothyroxinaemia during early pregnancy and subsequent child development: a 3-year follow-up study. *Clin Endocrinol (Oxf)* (2003) **59**:282–8. doi:10.1046/j.1365-2265.2003.01822.x
88. Vermiglio F, Lo Presti VP, Moleti M, Sidoti M, Tortorella G, Scaffidi G, et al. 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**:6054–60. doi:10.1210/jc.2004-0571
89. Morreale de Escobar G, Obregón MJ, Escobar del Rey F. Iodine deficiency and brain development in the first half of pregnancy. *Public Health Nutr* (2007) **10**:1554–70. doi:10.1017/S1368980007360928
90. Zimmermann MB, Jooste PL, Pandav CS. Iodine-deficiency disorders. *Lancet* (2008) **372**:1251–62. doi:10.1016/S0140-6736(08)61005-3
91. Andersson M, Aeberli I, Wüst N, Piacenza AM, Bucher T, Henschen I, et al. The Swiss iodized salt program provides adequate iodine for school children and pregnant women, but weaning infants not receiving iodine-containing complementary foods as well as their mothers are iodine deficient. *J Clin Endocrinol Metab* (2010) **95**:5217–24. doi:10.1210/jc.2010-0975
92. Santos NC, Costa P, Ruano D, Macedo A, Soares MJ, Valente J, et al. Revisiting thyroid hormones in schizophrenia. *J Thyroid Res* (2012) **2012**:569147. doi:10.1155/2012/569147
93. Bath SC, Steer CD, Golding J, Emmett P, Rayman MP. Effect of inadequate iodine status in UK pregnant women on cognitive outcomes in their children: results from the Avon longitudinal study of parents and children (ALSPAC). *Lancet* (2013) **382**:331–7. doi:10.1016/S0140-6736(13)60436-5
94. Hamza RT, Hewedi DH, Sallam MT. Iodine deficiency in Egyptian autistic children and their mothers: relation to disease severity. *Arch Med Res* (2013) **44**:555–61. doi:10.1016/j.arcmed.2013.09.012
95. Kooistra L, Crawford S, van Baar AL, Brouwers EP, Pop VJ. Neonatal effects of maternal hypothyroxinemia during early pregnancy. *Pediatrics* (2006) **117**:161–7. doi:10.1542/peds.2005-0227

96. Kasatkina EP, Samsonova LN, Ivakhnenko VN, Ibragimova GV, Ryabykh AV, Naumenko LL, et al. Gestational hypothyroxinemia and cognitive function in offspring. *Neurosci Behav Physiol* (2006) **36**:619–24. doi:10.1007/s11055-006-0066-0
97. Li Y, Shan Z, Tengm W, Yu X, Li Y, Fan C, et al. Abnormalities of maternal thyroid function during pregnancy affect neuropsychological development of their children at 25-30 months. *Clin Endocrinol (Oxf)* (2010) **72**:825–9. doi:10.1111/j.1365-2265.2009.03743.x
98. Berbel P, Mestre JL, Santamaría A, Palazón I, Franco A, Graells M, et al. 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**:511–9. doi:10.1089/thy.2008.0341
99. Suárez-Rodríguez M, Azcona-San Julián C, Alzina de Aguilar V. Hypothyroxinemia during pregnancy: the effect on neurodevelopment in the child. *Int J Dev Neurosci* (2012) **30**:435–8. doi:10.1016/j.ijdevneu.2012.07.004
100. Moreno-Reyes R, Suetens C, Mathieu F, Begaux F, Zhu D, Rivera MT, et al. Kashin-Beck osteoarthropathy in rural Tibet in relation to selenium and iodine status. *N Engl J Med* (1998) **339**:1112–20. doi:10.1056/NEJM199810153391604
101. Thilly CH, Swennen B, Bourdoux P, Ntambue K, Moreno-Reyes R, Gillies J, et al. The epidemiology of iodine-deficiency disorders in relation to goitrogenic factors and thyroid-stimulating-hormone regulation. *Am J Clin Nutr* (1993) **57**:267S–70S.
102. Kaas JH. Evolution of columns, modules, and domains in the neocortex of primates. *Proc Natl Acad Sci U S A* (2012) **109**:10655–60. doi:10.1073/pnas.1201892109
103. Luders E, Narr KL, Thompson PM, Rex DE, Jancke L, Steinmetz H, et al. Gender differences in cortical complexity. *Nat Neurosci* (2004) **7**:799–800. doi:10.1038/nn1277
104. Kaas JH. The evolution of brains from early mammals to humans. *Wiley Interdiscip Rev Cogn Sci* (2013) **4**:33–45. doi:10.1002/wcs.1206
105. Stenzel D, Huttner WB. Role of maternal thyroid hormones in the developing neocortex and during human evolution. *Front Neuroanat* (2013) **7**:19. doi:10.3389/fnana.2013.00019
106. Innocenti GM. Development and evolution: two determinants of cortical connectivity. *Prog Brain Res* (2011) **189**:65–75. doi:10.1016/B978-0-444-53884-0.00018-X
107. Amaral DG, Schumann CM, Nordahl CW. Neuroanatomy of autism. *Trends Neurosci* (2008) **31**:137–45. doi:10.1016/j.tins.2007.12.005
108. Coghlan S, Horder J, Inkster B, Mendez MA, Murphy DG, Nutt DJ. GABA system dysfunction in autism and related disorders: from synapse to symptoms. *Neurosci Biobehav Rev* (2012) **36**:2044–55. doi:10.1016/j.neubiorev.2012.07.005
109. Mountcastle V. The evolution of ideas concerning the function of the neocortex. *Cereb Cortex* (1995) **5**:289–95. doi:10.1093/cercor/5.4.289
110. Marín-Padilla M. Dual origin of the mammalian neocortex and evolution of the cortical plate. *Anat Embryol* (1978) **152**:109–26. doi:10.1007/BF00315920
111. Angevine JB Jr, Sidman RL. Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature* (1961) **192**:766–8. doi:10.1038/192766b0
112. Zecevic N, Rakic P. Development of layer I neurons in the primate cerebral cortex. *J Neurosci* (2001) **21**:5607–19.
113. Rakic P. Mode of cell migration to the superficial layers of fetal monkey neocortex. *J Comp Neurol* (1972) **145**:61–83. doi:10.1002/cne.901450105
114. Rakic P, Cameron RS, Komuro H. Recognition, adhesión, transmembrane signaling and cell motility in guided neuronal migration. *Curr Opin Neurobiol* (1994) **4**:63–9. doi:10.1016/0959-4388(94)90033-7
115. Rakic P. Specification of cerebral cortical areas. *Science* (1988) **241**:170–6. doi:10.1126/science.3291116
116. O'Leary DD, Sahara S. Genetic regulation of arealization of the neocortex. *Curr Opin Neurobiol* (2008) **18**:90–100. doi:10.1016/j.conb.2008.05.011
117. Krubitzer LA, Seelke AM. Cortical evolution in mammals: the bane and beauty of phenotypic variability. *Proc Natl Acad Sci U S A* (2012) **109**:10647–54. doi:10.1073/pnas.1201891109
118. Cholfin JA, Rubenstein JL. Frontal cortex subdivision patterning is coordinately regulated by Fgf8, Fgf17, and Emx2. *J Comp Neurol* (2008) **509**:144–55. doi:10.1002/cne.21709
119. Mallamaci A. Molecular bases of cortico-cerebral regionalization. *Prog Brain Res* (2011) **189**:37–64. doi:10.1016/B978-0-444-53884-0.00017-8
120. Caviness VS Jr, Rakic P. Mechanisms of cortical development: a view from mutations in mice. *Annu Rev Neurosci* (1978) **1**:297–326. doi:10.1146/annurev.ne.01.030178.001501
121. McConnell SK. Fates of visual cortical neurons in the ferret after isochronic and heterochronic transplantation. *J Neurosci* (1988) **8**:945–74.
122. Krubitzer L, Campi KL, Cooke DF. All rodents are not the same: a modern synthesis of cortical organization. *Brain Behav Evol* (2011) **78**:51–93. doi:10.1159/000327320
123. Kaas JH. The evolution of neocortex in primates. *Prog Brain Res* (2012) **195**:91–102. doi:10.1016/B978-0-444-53860-4.00005-2
124. O'Rourke NA, Dailey ME, Smith SJ, McConnell SK. Diverse migratory pathways in the developing cerebral cortex. *Science* (1992) **258**:299–302. doi:10.1126/science.1411527
125. Anderson SA, Eisenstat DD, Shi L, Rubenstein JL. Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. *Science* (1997) **278**:474–6. doi:10.1126/science.278.5337.474
126. Marin O. Cellular and molecular mechanisms controlling the migration of neocortical interneurons. *Eur J Neurosci* (2013) **38**:2019–29. doi:10.1111/ejn.12225
127. Letinic K, Rakic P. Telencephalic origin of human thalamic GABAergic neurons. *Nat Neurosci* (2001) **4**:931–6. doi:10.1038/nn0901-931
128. Letinic K, Zoncu R, Rakic P. Origin of GABAergic neurons in the human neocortex. *Nature* (2002) **417**:645–9. doi:10.1038/nature00779
129. Jones EG. The origins of cortical interneurons: mouse versus monkey and human. *Cereb Cortex* (2009) **19**:1953–6. doi:10.1093/cercor/bhp088
130. Le Magueresse C, Monyer H. GABAergic interneurons shape the functional maturation of the cortex. *Neuron* (2013) **77**:388–405. doi:10.1016/j.neuron.2013.01.011
131. Rubenstein JL. Annual research review: development of the cerebral cortex: implications for neurodevelopmental disorders. *J Child Psychol Psychiatry* (2011) **52**:339–55. doi:10.1111/j.1469-7610.2010.02307.x
132. Westerink RH, Beekwilder JP, Wadman WJ. Differential alterations of synaptic plasticity in dentate gyrus and CA1 hippocampal area of calbindin-D28K knockout mice. *Brain Res* (2012) **1450**:1–10. doi:10.1016/j.brainres.2012.02.036
133. Bayer SA, Altman J. *Neocortical Development*. New York, NY: Raven Press (1991).
134. Innocenti GM. Exuberant development of connections, and its possible permissive role in cortical evolution. *Trends Neurosci* (1995) **18**:397–402. doi:10.1016/0166-2236(95)93936-R
135. Rakic P. A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. *Trends Neurosci* (1995) **18**:383–8. doi:10.1016/0166-2236(95)93934-P
136. Obregon MJ, Mallol J, Pastor R, Morreale de Escobar G, Escobar del Rey F. L-thyroxine and 3,5,3'-triiodo-L-thyronine in rat embryos before onset of fetal thyroid function. *Endocrinology* (1984) **114**:305–7. doi:10.1210/endo-114-1-305
137. Kratzsch J, Pulzer F. Thyroid gland development and defects. *Best Pract Res Clin Endocrinol Metab* (2008) **22**:57–75. doi:10.1016/j.beem.2007.08.006
138. Biebermann H, Grüters A, Schöneberg T, Gudermann T. Congenital hypothyroidism caused by mutations in the thyrotropin-receptor gene. *N Engl J Med* (1997) **336**:1390–1. doi:10.1056/NEJM199705083361914
139. Pasca di Magliano M, Di Lauro R, Zannini M. Pax8 has a key role in thyroid cell differentiation. *Proc Natl Acad Sci U S A* (2000) **97**:13144–9. doi:10.1073/pnas.240336397
140. 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**:4036–43. doi:10.1210/en.2006-0390
141. Friesema EC, Grueters A, Biebermann H, Krude H, von Moers A, Reeser M, et al. Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet* (2004) **364**:1435–7. doi:10.1016/S0140-6736(04)17226-7
142. Trajkovic M, Visser TJ, Mittag J, Horn S, Lukas J, Darras VM, et al. Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8. *J Clin Invest* (2007) **117**:627–35. doi:10.1172/JCI28253
143. Braun D, Wirth EK, Wohlgemuth F, Reix N, Klein MO, Gruters A, et al. Aminoaciduria, but normal thyroid hormone levels and signalling, in mice lacking the amino acid and thyroid hormone transporter Slc7a8. *Biochem J* (2011) **439**:249–55. doi:10.1042/BJ20110759

144. Allan W, Herndon CN, Dudley FC. Some examples of the inheritance of mental deficiency: apparently sex-linked idiocy and microcephaly. *Am J Ment Defic* (1944) **48**:325–34.
145. 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**:168–75. doi:10.1086/380999
146. Wirth EK, Roth S, Blechschmidt C, Hölter SM, Becker L, Racz I, et al. 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**:9439–49. doi:10.1523/JNEUROSCI.6055-08.2009
147. 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**:476–84. doi:10.1172/JCI26240
148. Hernandez A, Morte B, Belinchón 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**:2919–28. doi:10.1210/en.2011-1905
149. Morte B, Ceballos A, Diez D, Grijota-Martínez C, Dumitrescu AM, Di Cosmo C, et al. 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**:2381–7. doi:10.1210/en.2009-0944
150. Flamant F, Samarut J. Thyroid hormone receptors: lessons from knockout and knock-in mutant mice. *Trends Endocrinol Metab* (2003) **14**:85–90. doi:10.1016/S1043-2760(02)00043-7
151. Refetoff S, DeWind LT, DeGroot LJ. Familial syndrome combining deaf-mutism, stuppled epiphyses, goiter and abnormally high PBI: possible target organ refractoriness to thyroid hormone. *J Clin Endocrinol Metab* (1967) **27**:279–94. doi:10.1210/jcem-27-2-279
152. Beck-Peccoz P, Chatterjee VK. The variable clinical phenotype in thyroid hormone resistance syndrome. *Thyroid* (1994) **4**:225–32. doi:10.1089/thy.1994.4.225
153. Rosenberg IN. The antithyroid activity of some compounds that inhibit peroxidase. *Science* (1952) **116**:503–5. doi:10.1126/science.116.3019.503
154. Escobar del Rey F, Morreale de Escobar G. The effect of propylthiouracil, methylthiouracil and thiouracil on the peripheral metabolism of 1-thyroxine in thyroidectomized, 1-thyroxine maintained rats. *Endocrinology* (1961) **69**:456–65. doi:10.1210/endo-69-3-456
155. De Groot LJ, Davis AM. Studies on the biosynthesis of iodotyrosines: a soluble thyroidal iodide-peroxidase tyrosine-iodinase system. *Endocrinology* (1962) **70**:492–504. doi:10.1210/endo-70-4-492
156. Oppenheimer JH, Schwartz H, Surks L. MIPropylthiouracil inhibits the conversion of L-thyroxine to L-triiodothyronine. An explanation of the antithyroxine effect of propylthiouracil and evidence supporting the concept that triiodothyronine is the active thyroid hormone. *J Clin Invest* (1972) **51**:2493–7. doi:10.1172/JCI107063
157. Azizi F, Amouzegar A. Management of hyperthyroidism during pregnancy and lactation. *Eur J Endocrinol* (2011) **164**:871–6. doi:10.1530/EJE-10-1030
158. Ausó E, Lavado-Autric R, Cuevas E, Escobar del Rey F, 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**:4037–47. doi:10.1210/en.2004-0274
159. Cuevas E, Ausó E, Telefont M, Morreale de Escobar G, Sotelo C, Berbel P. Transient maternal hypothyroxinemia at onset of corticogenesis alters tangential migration of medial ganglionic eminence-derived neurons. *Eur J Neurosci* (2005) **22**:541–51. doi:10.1111/j.1460-9568.2005.04243.x
160. Mano MT, Potter BJ, Belling GB, Chavadej J, Hetzel BS. Fetal brain in response to iodine deficiency in a primate model (*Callithrix jacchus*). *J Neurol Sci* (1987) **9**:287–300. doi:10.1016/0022-510X(87)90236-X
161. Potter BJ, Mano MT, Belling GB, McIntosh GH, Hua C, Cragg BG, et al. Retarded fetal brain development resulting from severe dietary iodine deficiency in sheep. *Neuropathol Appl Neurobiol* (1982) **8**:303–13. doi:10.1111/j.1365-2990.1982.tb00299.x
162. Li JQ, Wang X, Yan YQ, Wang KW, Qin DK, Xin ZF, et al. The effects on fetal brain development in the rat of a severely iodine deficient diet derived from an endemic area: observations on the first generation. *Neuropathol Appl Neurobiol* (1986) **12**:261–76.
163. Martínez-Galán JR, Pedraza P, Santacana M, Escobar del Rey 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**:2701–9. doi:10.1172/JCI119459
164. Lavado-Autric R, Ausó E, García-Velasco JV, Arufe M, Escobar del Rey F, Berbel P, et al. Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. *J Clin Invest* (2003) **111**:1073–82. doi:10.1172/JCI200316262
165. Yu F, Wang Y, Xu H, Dong J, Wei W, Wang Y, et al. Developmental iodine deficiency delays the maturation of newborn granule neurons associated with downregulation of p35 in postnatal rat hippocampus. *Environ Toxicol* (2012) **29**:847–55. doi:10.1002/tox.21811
166. Wang Y, Wei W, Wang Y, Dong J, Song B, Min H, et al. Neurotoxicity of developmental hypothyroxinemia and hypothyroidism in rats: impairments of long-term potentiation are mediated by phosphatidylinositol 3-kinase signaling pathway. *Toxicol Appl Pharmacol* (2013) **271**:257–65. doi:10.1016/j.taap.2013.04.034
167. Scow RW, Simpson ME. Thyroidectomy in the newborn rat. *Anat Rec* (1945) **91**:209–26. doi:10.1002/ar.1090910305
168. Morreale de Escobar G, Obregon MJ, Escobar del Rey F. Fetal and maternal thyroid hormones. *Horm Res* (1987) **26**:12–27. doi:10.1159/000180681
169. Berbel P, Navarro D, Ausó E, Varea E, Rodríguez AE, Ballesta JJ, et al. Role of late maternal thyroid hormones in cerebral cortex development: an experimental model for human prematurity. *Cereb Cortex* (2010) **20**:1462–75. doi:10.1093/cercor/bhp212
170. Dowling AL, Martz GU, Leonard JL, Zoeller RT. Acute changes in maternal thyroid hormone induce rapid and transient changes in gene expression in fetal rat brain. *J Neurosci* (2000) **20**:2255–65.
171. Dowling AL, Zoeller RT. Thyroid hormone of maternal origin regulates the expression of RC3/neurogranin mRNA in the fetal rat brain. *Brain Res Mol Brain Res* (2000) **82**:126–32. doi:10.1016/S0169-328X(00)00190-X
172. Alvarez-Dolado M, Ruiz M, Del Rio JA, Alcantara S, Burgaya F, Sheldon M. Thyroid hormone regulates reelin and dab-1 expression during brain development. *J Neurosci* (1999) **19**:6979–93.
173. Pathak A, Sinha RA, Mohan V, Mitra K, Godbole MM. Maternal thyroid hormone before the onset of fetal thyroid function regulates reelin and downstream signaling cascade affecting neocortical neuronal migration. *Cereb Cortex* (2011) **21**:11–21. doi:10.1093/cercor/bhq052
174. Miller LD, Park KS, Guo QM, Alkharouf NW, Malek RL, Lee NH, et al. Silencing of Wnt signaling and activation of multiple metabolic pathways in response to thyroid hormone-stimulated cell proliferation. *Mol Cell Biol* (2001) **21**:6626–39. doi:10.1128/MCB.21.19.6626-6639.2001
175. Matthews RP, Guthrie CR, Wailes LM, Zhao X, Means AR, McKnight GS. Calcium/calmodulin-dependent protein kinase types II and IV differentially regulate CREB-dependent gene expression. *Mol Cell Biol* (1994) **14**:6107–16. doi:10.1128/MCB.14.9.6107
176. Watterson DM, Mirzoeva S, Guo L, Whyte A, Bourguignon JJ, Hibert M, et al. Ligand modulation of glial activation: cell permeable, small molecule inhibitors of serine-threonine protein kinases can block induction of interleukin 1 beta and nitric oxide synthase II. *Neurochem Int* (2001) **39**:459–68. doi:10.1016/S0197-0186(01)00053-5
177. Murray PD, Kingsbury TJ, Krueger BK. Failure of Ca2+-activated, CREB-dependent transcription in astrocytes. *Glia* (2009) **57**:828–34. doi:10.1002/glia.20809
178. Kuno-Murata M, Koibuchi N, Fukuda H, Murata M, Chin WW. Augmentation of thyroid hormone receptor-mediated transcription by Ca2+/calmodulin-dependent protein kinase type IV. *Endocrinology* (2000) **141**:2275–8. doi:10.1210/endo.141.6.7612
179. McKenzie GJ, Stevenson P, Ward G, Papadia S, Bading H, Chawla SJ, et al. Nuclear Ca2+ and CaM kinase IV specify hormonal- and Notch-responsiveness. *J Neurochem* (2005) **93**:171–85. doi:10.1111/j.1471-4159.2005.03010.x
180. Navarro D, Alvarado M, Navarrete F, Giner M, Pacheco P, Morreale de Escobar G, et al. Maternal and fetal rat's hypothyroidism during gestation and lactation unbalances cortical VGluT1-VGAT immunoreactivity and alters attention déficit and anxiety. *Eur Thyroid J* (2012) **1**(Suppl 1):208. doi:10.1159/000339890
181. Carlezon WA Jr, Duman RS, Nestler EJ. The many faces of CREB. *Trends Neurosci* (2005) **28**:436–45. doi:10.1016/j.tins.2005.06.005

182. Qiu Z, Sylwestrak EL, Lieberman DN, Zhang Y, Liu XY, Ghosh A. The Rett syndrome protein MeCP2 regulates synaptic scaling. *J Neurosci* (2012) **32**:989–94. doi:10.1523/JNEUROSCI.0175-11.2012
183. Lv J, Xin Y, Zhou W, Qiu Z. The epigenetic switches for neural development and psychiatric disorders. *J Genet Genomics* (2013) **40**:339–46. doi:10.1016/j.jgg.2013.04.007
184. Wang H, Fukushima H, Kida S, Zhuo M. Ca2+/calmodulin-dependent protein kinase IV links group I metabotropic glutamate receptors to fragile X mental retardation protein in cingulate cortex. *J Biol Chem* (2009) **284**:18953–62. doi:10.1074/jbc.M109.019141
185. Wang H, Morishita Y, Miura D, Naranjo JR, Kida S, Zhuo M. Roles of CREB in the regulation of FMRP by group I metabotropic glutamate receptors in cingulate cortex. *Mol Brain* (2012) **5**:27. doi:10.1186/1756-6606-5-27
186. Krueger DD, Bear MF. Toward fulfilling the promise of molecular medicine in fragile X syndrome. *Annu Rev Med* (2011) **62**:411–29. doi:10.1146/annurev-med-061109-134644
187. Tuchman R, Hirtz D, Mamounas LA. NINDS epilepsy and autism spectrum disorders workshop report. *Neurology* (2013) **29**:1630–6. doi:10.1212/WNL.0b013e3182a9f482
188. Lu J, Wu Y, Sousa N, Almeida OF. SMAD pathway mediation of BDNF and TGF beta 2 regulation of proliferation and differentiation of hippocampal granule neurons. *Development* (2005) **132**:3231–42. doi:10.1242/dev.01893
189. Castrén ML, Castrén E. BDNF in fragile X syndrome. *Neuropharmacology* (2014) **76**(Pt C):729–36. doi:10.1016/j.neuropharm.2013.05.018
190. Eayrs JT, Taylor SH. The effect of thyroid deficiency induced by methyl thiouracil on the maturation of the central nervous system. *J Anat* (1951) **85**:350–8.
191. Mohan V, Sinha RA, Pathak A, Rastogi L, Kumar P, Pal A, et al. Maternal thyroid hormone deficiency affects the fetal neocorticogenesis by reducing the proliferating pool, rate of neurogenesis and indirect neurogenesis. *Exp Neurol* (2012) **237**:477–88. doi:10.1016/j.expneurol.2012.07.019
192. Berbel P, Ausó E, Garcia-Velasco JV, Molina ML, Camacho M. Role of thyroid hormones in the maturation and organisation of rat barrel cortex. *Neuroscience* (2001) **107**:383–94. doi:10.1016/S0306-4522(01)00368-2
193. Rami A, Rabié A, Patel AJ. Thyroid hormone and development of the rat hippocampus: cell acquisition in the dentate gyrus. *Neuroscience* (1986) **19**:1207–16. doi:10.1016/0306-4522(86)90134-X
194. Perez-Juste G, Aranda A. The cyclin-dependent kinase inhibitor p27(Kip1) is involved in thyroid hormone-mediated neuronal differentiation. *J Biol Chem* (1999) **274**:5026–31. doi:10.1074/jbc.274.8.5026
195. Qiu R, Wang J, Tsark W, Lu Q. Essential role of PDZ-RGS3 in the maintenance of neural progenitor cells. *Stem Cells* (2010) **28**:1602–10. doi:10.1002/stem.478
196. O'Roak BJ, Vives L, Fu W, Egertson JD, Stanaway IB, Phelps IG, et al. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. *Science* (2012) **338**:1619–22. doi:10.1126/science.1227764
197. Barr AR, Kilmartin JV, Gergely F. CDK5RAP2 functions in centrosome to spindle pole attachment and DNA damage response. *J Cell Biol* (2010) **189**:23–39. doi:10.1083/jcb.200912163
198. Wang Y, Wang Y, Dong J, Wei W, Song B, Min H, et al. Developmental hypothyroxinemia and hypothyroidism reduce proliferation of cerebellar granule neuron precursors in rat offspring by downregulation of the sonic hedgehog signaling pathway. *Mol Neurobiol* (2014) **49**:1143–52. doi:10.1007/s12035-013-8587-3
199. Kuida K, Haydar TF, Kuan CY, Gu Y, Taya C, Karasuyama H, et al. Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. *Cell* (1998) **94**:325–37. doi:10.1016/S0092-8674(00)81476-2
200. Chenn A, Walsh CA. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. *Science* (2002) **297**:365–9. doi:10.1126/science.1074192
201. Singh R, Upadhyay G, Godbole MM. Hypothyroidism alters mitochondrial morphology and induces release of apoptogenic proteins during rat cerebellar development. *J Endocrinol* (2003) **176**:321–9. doi:10.1677/joe.0.1760321
202. Babu S, Sinha RA, Mohan V, Rao G, Pal A, Pathak A, et al. Effect of hypothyroxinemia on thyroid hormone responsiveness and action during rat postnatal neocortical development. *Exp Neurol* (2011) **228**:91–8. doi:10.1016/j.expneurol.2010.12.012
203. Sugimoto C, Maekawa S, Miyata S. OBCAM, an immunoglobulin superfamily cell adhesion molecule, regulates morphology and proliferation of cerebral astrocytes. *J Neurochem* (2010) **112**:818–28. doi:10.1111/j.1471-4159.2009.06513.x
204. Martínez-Galán JR, Escobar del Rey F, Morreale de Escobar G, Santacana M, Ruíz-Marcos A. Hypothyroidism alters the development of radial glial cells in the term fetal and postnatal neocortex of the rat. *Brain Res Dev Brain Res* (2004) **153**:109–14. doi:10.1016/j.devbrainres.2004.08.002
205. Lucio RA, García JV, Ramón Cerezo J, Pacheco P, Innocenti GM, Berbel P. The development of auditory callosal connections in normal and hypothyroid rats. *Cereb Cortex* (1997) **7**:303–16. doi:10.1093/cercor/7.4.303
206. 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**:2593–7. doi:10.1210/en.2006-1276
207. Tan XJ, Fan XT, Kim HJ, Butler R, Webb P, Warner M, et al. Liver X receptor beta and thyroid hormone receptor alpha in brain cortical layering. *Proc Natl Acad Sci U S A* (2010) **107**:12305–10. doi:10.1073/pnas.1006162107
208. Fan X, Kim HJ, Bouton D, Warner M, Gustafsson JA. Expression of liver X receptor beta is essential for formation of superficial cortical layers and migration of later-born neurons. *Proc Natl Acad Sci U S A* (2008) **105**:13445–50. doi:10.1073/pnas.0806974105
209. Guo L, Xu P, Tang X, Wu Q, Xing Y, Gustafsson JA, et al. Liver X receptor β delays transformation of radial glial cells into astrocytes during mouse cerebral cortical development. *Neurochem Int* (2014) **71**:8–16. doi:10.1016/j.neuint.2014.03.009
210. Berkenstam A, Färnegårdh M, Gustafsson JA. Convergence of lipid homeostasis through liver X and thyroid hormone receptors. *Mech Ageing Dev* (2004) **125**:707–17. doi:10.1016/j.mad.2004.05.005
211. Berbel P, Marco P, Cerezo JR, DeFelipe J. Distribution of parvalbumin immunoreactivity in the neocortex of hypothyroid adult rats. *Neurosci Lett* (1996) **204**:65–8. doi:10.1016/0304-3940(96)12318-1
212. Morte B, Manzano J, Scanlan T, Vennström 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**:3985–9. doi:10.1073/pnas.062413299
213. Ceballos A, Belinchon MM, Sanchez-Mendoza E, Grijota-Martinez C, Dumitrescu AM, Refetoff S, et al. Importance of monocarboxylate transporter 8 for the blood-brain barrier-dependent availability of 3,5,3'-triiodo-L-thyronine. *Endocrinology* (2009) **150**:2491–6. doi:10.1210/en.2008-1616
214. Nicholson JL, Altman J. The effects of early hypo- and hyperthyroidism on the development of the rat cerebellar cortex. Synaptogenesis in the molecular layer. *Brain Res* (1972) **44**:25–36. doi:10.1016/0006-8993(72)90362-9
215. Lauder JM. Thyroid influences on the developing cerebellum and hippocampus of the rat. In: DeLong GR, Robbins J, Condliffe PG, editors. *Iodine and the Brain.* New York, NY: Plenum Press (1989). p. 79–90.
216. Berbel P, Guadaño-Ferraz A, Martínez M, Quiles JA, Balboa R, Innocenti GM. Organization of auditory callosal connections in hypothyroid rats. *Eur J Neurosci* (1993) **5**:1465–78. doi:10.1111/j.1460-9568.1993.tb00214.x
217. Gilbert ME, Sui L, Walker MJ, Anderson W, Thomas S, Smoller SN, et al. Thyroid hormone insufficiency during brain development reduces parvalbumin immunoreactivity and inhibitory function in the hippocampus. *Endocrinology* (2007) **148**:92–102. doi:10.1210/en.2006-0164
218. Wallis K, Sjögren M, van Hogerlinden M, Silberberg G, Fisahn A, Nordström K, et al. Locomotor deficiencies and aberrant development of subtype-specific GABAergic interneurons caused by an unliganded thyroid hormone receptor alpha1. *J Neurosci* (2008) **28**:1904–15. doi:10.1523/JNEUROSCI.5163-07.2008
219. Venero C, Guadaño-Ferraz A, Herrero AI, Nordström K, Manzano J, de Escobar GM, et al. Anxiety, memory impairment, and locomotor dysfunction caused by a mutant thyroid hormone receptor alpha1 can be ameliorated by T3 treatment. *Genes Dev* (2005) **19**:2152–63. doi:10.1101/gad.346105
220. Patel LS, Wenzel HJ, Schwartzkroin PA. Physiological and morphological characterization of dentate granule cells in the p35 knock-out mouse hippocampus: evidence for an epileptic circuit. *J Neurosci* (2004) **24**:9005–14. doi:10.1523/JNEUROSCI.2943-04.2004
221. Van Middlesworth L, Norris CH. Audiogenic seizures and cochlear damage in rats after perinatal antithyroid treatment. *Endocrinology* (1980) **106**:1686–90. doi:10.1210/endo-106-6-1686
222. Eayrs JT, Goohead B. Postnatal development of the cerebral cortex in the rat. *J Anat* (1959) **93**:385–402.

223. Vincent J, Legrand C, Rabié A, Legrand J. Effects of thyroid hormone on synaptogenesis in the molecular layer of the developing rat cerebellum. *J Physiol* (1982/1983) **78**:729–38.
224. Yu X, Malenka RC. Beta-catenin is critical for dendritic morphogenesis. *Nat Neurosci* (2003) **6**:1169–77. doi:10.1038/nn1132
225. 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**:147–61. doi:10.1002/cne.902910109
226. Gravel C, Hawkes R. Maturation of the corpus callosum of the rat: I. Influence of thyroid hormones on the topography of callosal projections. *J Comp Neurol* (1990) **291**:128–46. doi:10.1002/cne.902910109
227. Guadaño-Ferraz A, Escobar del Rey F, Morreale de Escobar G, Innocenti GM, Berbel P. The development of the anterior commissure in normal and hypothyroid rats. *Brain Res Dev Brain Res* (1994) **81**:293–308. doi:10.1016/0165-3806(94)90315-8
228. Berbel P, Guadaño-Ferraz A, Angulo A, Ramón Cerezo J. Role of thyroid hormones in the maturation of interhemispheric connections in rats. *Behav Brain Res* (1994) **64**:9–14. doi:10.1016/0166-4328(94)90114-7
229. Nunez J, Couchie D, Aniello F, Bridoux AM. Regulation by thyroid hormone of microtubule assembly and neuronal differentiation. *Neurochem Res* (1991) **16**:975–82. doi:10.1007/BF00965840
230. Muñoz A, Rodriguez-Peña A, Perez-Castillo A, Ferreiro B, Sutcliffe JG, Bernal J. Effects of neonatal hypothyroidism on rat brain gene expression. *Mol Endocrinol* (1991) **5**:273–80. doi:10.1210/mend-5-2-273
231. Li CP, Olavarria JF, Greger BE. Occipital cortico-pyramidal projection in hypothyroid rats. *Brain Res Dev Brain Res* (1995) **89**:227–34. doi:10.1016/0165-3806(95)00119-X
232. Innocenti GM, Price DJ. Exuberance in the development of cortical networks. *Nat Rev Neurosci* (2005) **6**:955–65. doi:10.1038/nrn1790
233. Friauf E, McConnell SK, Shatz CJ. Functional synaptic circuits in the subplate during fetal and early postnatal development of cat visual cortex. *J Neurosci* (1990) **10**:2601–13.
234. Oeschger FM, Wang WZ, Lee S, García-Moreno F, Goffinet AM, Arbonés ML, et al. Gene expression analysis of the embryonic subplate. *Cereb Cortex* (2012) **22**:1343–59. doi:10.1093/cercor/bhr197
235. Torres-Reveron J, Friedlander MJ. Properties of persistent postnatal cortical subplate neurons. *J Neurosci* (2007) **27**:9962–74. doi:10.1523/JNEUROSCI.1536-07.2007
236. Kanold PO, Kara P, Reid RC, Shatz CJ. Role of subplate neurons in functional maturation of visual cortical columns. *Science* (2003) **301**:521–5. doi:10.1126/science.1084152
237. Kanold PO, Shatz CJ. Subplate neurons regulate maturation of cortical inhibition and outcome of ocular dominance plasticity. *Neuron* (2006) **51**:627–38. doi:10.1016/j.neuron.2006.07.008
238. Piñon MC, Jethwa A, Jacobs E, Campagnoni A, Molnár Z. Dynamic integration of subplate neurons into the cortical barrel field circuitry during postnatal development in the Golli-tau-eGFP (GTE) mouse. *J Physiol* (2009) **587**:1903–15. doi:10.1113/jphysiol.2008.167767
239. Navarro D, Alvarado M, Morte B, Berbel D, Sesma J, Pacheco P, et al. Late maternal hypothyroidism alters the expression of Camk4 in neocortical subplate neurons. A comparison with Nurr1 labeling. *Cereb Cortex* (2013). doi:10.1093/cercor/bht129
240. Volpe JJ. Subplate neurons – missing link in brain injury of the premature infant? *Pediatrics* (1996) **97**:112–3.
241. Volpe JJ. Electroencephalography may provide insight into timing of premature brain injury. *Pediatrics* (2009) **124**:e542–4. doi:10.1542/peds.2009-1244
242. Eastwood SL, Harrison PJ. Cellular basis of reduced cortical reelin expression in schizophrenia. *Am J Psychiatry* (2006) **163**:540–2. doi:10.1176/appi.ajp.163.3.540
243. McQuillen PS, Ferriero DM. Perinatal subplate neuron injury: implications for cortical development and plasticity. *Brain Pathol* (2005) **15**:250–60. doi:10.1111/j.1750-3639.2005.tb00528.x
244. Stolp H, Neuhaus A, Sundramoorthi R, Molnár Z. The long and the short of it: gene and environment interactions during early cortical development and consequences for long-term neurological disease. *Front Psychiatry* (2012) **3**:50. doi:10.3389/fpsyt.2012.00050
245. Hoerder-Suabedissen A, Oeschger FM, Krishnan ML, Belgard TG, Wang WZ, Lee S, et al. Expression profiling of mouse subplate reveals a dynamic gene network and disease association with autism and schizophrenia. *Proc Natl Acad Sci U S A* (2013) **110**:3555–60. doi:10.1073/pnas.1218510110
246. Ausó E, Cases O, Fouquet C, Camacho M, García-Velasco JV, Gaspar P, et al. Protracted expression of serotonin transporter and altered thalamocortical projections in the barrelfield of hypothyroid rats. *Eur J Neurosci* (2001) **14**:1968–80. doi:10.1046/j.0953-816x.2001.01815.x
247. Blue ME, Erzurumlu RS, Jhaveri S. A comparison of pattern formation by thalamocortical and serotonergic afferents in the rat barrel field cortex. *Cereb Cortex* (1991) **1**:380–9. doi:10.1093/cercor/1.5.380
248. Osterheld-Haas MC, Hornung JP. Laminar development of the mouse barrel cortex: effects of neurotoxins against monoamines. *Exp Brain Res* (1996) **110**:183–95. doi:10.1007/BF00228550
249. Bennett-Clarke CA, Leslie MJ, Lane RD, Rhoades RW. Effect of serotonin depletion on vibrissa-related patterns of thalamic afferents in the rat's somatosensory cortex. *J Neurosci* (1994) **14**:7594–607.
250. Sanchez-Toscano F, Escobar del Rey F, Morreale de Escobar G, Ruiz-Marcos A. Measurement of the effects of hypothyroidism on the number and distribution of spines along the apical shaft of pyramidal neurons of the rat cerebral cortex. *Brain Res* (1977) **126**:547–50. doi:10.1016/0006-8993(77)90606-0
251. Wei W, Wang Y, Wang Y, Dong J, Min H, Song B, et al. Developmental hypothyroxinaemia induced by maternal mild iodine deficiency delays hippocampal axonal growth in the rat offspring. *J Neuroendocrinol* (2013) **25**:852–62. doi:10.1111/jne.12058
252. Berbel P, Innocenti GM. The development of the corpus callosum in cats: a light- and electron-microscopic study. *J Comp Neurol* (1988) **276**:132–56. doi:10.1002/cne.902760109
253. Savage DD, Otero MA, Montano CY, Razani-Boroujerdi S, Paxton LL, Kasarskis EJ. Perinatal hypothyroidism decreases hippocampal mossy fiber zinc density in rats. *Neuroendocrinology* (1992) **55**:20–7. doi:10.1159/000126092
254. Madeira MD, Paula-Barbosa MM. Reorganization of mossy fiber synapses in male and female hypothyroid rats: a stereological study. *J Comp Neurol* (1993) **337**:334–52. doi:10.1002/cne.903370213
255. Melo CV, Mele M, Curcio M, Comprido D, Silva CG, Duarte CB. BDNF regulates the expression and distribution of vesicular glutamate transporters in cultured hippocampal neurons. *PLoS One* (2013) **8**(1):e53793. doi:10.1371/journal.pone.0053793
256. Chakraborty G, Magagna-Poveda A, Parratt C, Umans JG, MacLusky NJ. Reduced hippocampal brain-derived neurotrophic factor (BDNF) in neonatal rats after prenatal exposure to propylthiouracil (PTU). *Endocrinology* (2012) **153**:1311–6. doi:10.1210/en.2011-1437
257. Wong CC, Leung MS. Effects of neonatal hypothyroidism on the expressions of growth cone proteins and axon guidance molecules related genes in the hippocampus. *Mol Cell Endocrinol* (2001) **184**:143–50. doi:10.1016/S0303-7207(01)00592-5
258. Koibuchi N, Chin WW. Thyroid hormone action and brain development. *Trends Endocrinol Metab* (2000) **11**:123–8. doi:10.1016/S1043-2760(00)00238-1
259. Cortés C, Eugenin E, Aliaga E, Carreño LJ, Bueno SB, Gonzalez P, et al. Hypothyroidism in the adult rat causes an incremento of Bdnf in the brain, neuronal and astrocytes apoptosis, gliosis and deterioration of the postsunaptic density. *Thyroid* (2012) **22**:951–63. doi:10.1089/thy.2010.0400
260. Sajdel-Sulkowska EM, Xu M, Koibuchi N. Increase in cerebellar neurotrophin-3 and oxidative stress markers in autism. *Cerebellum* (2009) **8**:366–72. doi:10.1007/s12311-009-0105-9
261. Sánchez-Ponce D, DeFelipe J, Garrido JJ, Muñoz A. In vitro maturation of the cisternal organelle in the hippocampal neuron's axon initial segment. *Mol Cell Neurosci* (2011) **48**:104–16. doi:10.1016/j.mcn.2011.06.010
262. Bridi MS, Abel T. The NR4A orphan nuclear receptors mediate transcription-dependent hippocampal synaptic plasticity. *Neurobiol Learn Mem* (2013) **105**:151–8. doi:10.1016/j.nlm.2013.06.020
263. Modregger J, Ritter B, Witter B, Paulsson M, Plomann M. All three PACSIN isoforms bind to endocytic proteins and inhibit endocytosis. *J Cell Sci* (2000) **113**:4511–21.
264. Gilbert ME, Sui L. Dose-dependent reductions in spatial learning and synaptic function in the dentate gyrus of adult rats following developmental thyroid hormone insufficiency. *Brain Res* (2006) **1069**:10–22. doi:10.1016/j.brainres.2005.10.049
265. Alzoubi KH, Gerges NZ, Aleisa AM, Alkadhi KA. Levothyroxin restores hypothyroidism-induced impairment of hippocampus-dependent learning

and memory: behavioral, electrophysiological, and molecular studies. *Hippocampus* (2009) **19**:66–78. doi:10.1002/hipo.20476
266. Opazo MC, Gianini A, Pancetti F, Azkcona G, Alarcón L, Lizana R, et al. Maternal hypothyroxinemia impairs spatial learning and synaptic nature and function in the offspring. *Endocrinology* (2008) **149**:5097–106. doi:10.1210/en.2008-0560
267. Sánchez-Alonso JL, Muñoz-Cuevas J, Vicente-Torres MA, Colino A. Role of low-voltage-activated calcium current on the firing pattern alterations induced by hypothyroidism in the rat hippocampus. *Neuroscience* (2010) **171**:993–1005. doi:10.1016/j.neuroscience.2010.10.003
268. Caminiti R, Ghaziri H, Galuske R, Hof PR, Innocenti GM. Evolution amplified processing with temporally dispersed slow neuronal connectivity in primates. *Proc Natl Acad Sci U S A* (2009) **106**:19551–6. doi:10.1073/pnas.0907655106
269. Ng L, Pedraza PE, Faris JS, Vennström B, Curran T, Morreale de Escobar G, et al. Audiogenic seizure susceptibility in thyroid hormone receptor beta-deficient mice. *Neuroreport* (2001) **12**:2359–62. doi:10.1097/00001756-200108080-00015
270. Negishi T, Kawasaki K, Sekiguchi S, Ishii Y, Kyuwa S, Kuroda Y, et al. Attention-deficit and hyperactive neurobehavioural characteristics induced by perinatal hypothyroidism in rats. *Behav Brain Res* (2005) **159**:323–31. doi:10.1016/j.bbr.2004.11.012
271. Gilbert ME, Lasley SM. Developmental thyroid hormone insufficiency and brain development: a role for brain-derived neurotrophic factor (BDNF)? *Neuroscience* (2013) **239**:253–70. doi:10.1016/j.neuroscience.2012.11.022
272. Suo Z, Cox AA, Bartelli N, Rasul I, Festoff BW, Premont RT, et al. GRK5 deficiency leads to early Alzheimer-like pathology and working memory impairment. *Neurobiol Aging* (2007) **28**:1873–88. doi:10.1016/j.neurobiolaging.2006.08.013
273. Rao JS, Rapoport SI, Kim HW. Decreased GRK3 but not GRK2 expression in frontal cortex from bipolar disorder patients. *Int J Neuropsychopharmacol* (2009) **12**:851–60. doi:10.1017/S146114570900025X
274. Wang L, Zhou C, Wang Z, Liu J, Jing Z, Zhang Z, et al. Dynamic variation of genes profiles and pathways in the hippocampus of ischemic mice: a genomic study. *Brain Res* (2011) **1372**:13–21. doi:10.1016/j.brainres.2010.11.099
275. Klugmann M, Leichtlein CB, Symes CW, Klaussner BC, Brooks AI, Young D, et al. A novel role of circadian transcription factor DBP in hippocampal plasticity. *Mol Cell Neurosci* (2006) **31**:303–14. doi:10.1016/j.mcn.2005.09.019
276. Bailey A, Luthert P, Dean A, Harding B, Janota I, Montgomery M, et al. A clinicopathological study of autism. *Brain* (1998) **121**:889–905. doi:10.1093/brain/121.5.889
277. Palmen SJ, van Engeland H, Hof PR, Schmitz C. Neuropathological findings in autism. *Brain* (2004) **127**:2572–83. doi:10.1093/brain/awh287
278. Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Marchi E, et al. The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. *Acta Neuropathol* (2010) **119**:755–70. doi:10.1007/s00401-010-0655-4
279. Castermans D, Vermeesch JR, Fryns JP, Steyaert JG, Van de Ven WJ, Creemers JW, et al. Identification and characterization of the TRIP8 and REEP3 genes on chromosome 10q21.3 as novel candidate genes for autism. *Eur J Hum Genet* (2007) **15**:422–31. doi:10.1038/sj.ejhg.5201785
280. Courchesne E, Pierce K, Schumann CM, Redcay E, Buckwalter JA, Kennedy DP, et al. Mapping early brain development in autism. *Neuron* (2007) **56**:399–413. doi:10.1016/j.neuron.2007.10.016
281. Kern JK, Geier DA, Sykes LK, Geier MR. Evidence of neurodegeneration in autism spectrum disorder. *Transl Neurodegener* (2013) **2**:17. doi:10.1186/2047-9158-2-17
282. Petropoulos H, Friedman SD, Shaw DWW, Artru AA, Dawson G, Dager SR. Gray matter abnormalities in autism spectrum disorder revealed by T2 relaxation. *Neurology* (2006) **67**:632–6. doi:10.1212/01.wnl.0000229923.08213.1e
283. Hardan AY, Girgis RR, Lacerda ALT, Yorbik O, Kilpatrick M, Keshavan MS. Magnetic resonance imaging study of the orbitofrontal cortex in autism. *J Child Neurol* (2006) **21**:866–71. doi:10.1177/08830738060210100701
284. Courchesne E, Mouton PR, Calhoun ME, Semendeferi K, Ahrens-Barbeau C, Hallet MJ, et al. Neuron number and size in prefrontal cortex of children with autism. *JAMA* (2011) **306**:2001–10. doi:10.1001/jama.2011.1638
285. McAlonan GM, Cheung V, Cheung C, Suckling J, Lam GY, Tai KS, et al. Mapping the brain in autism. A voxel-based MRI study of volumetric differences and intercorrelations in autism. *Brain* (2005) **128**:268–76. doi:10.1093/brain/awh332
286. Theoharides TC, Asadi S, Patel AB. Focal brain inflammation and autism. *J Neuroinflammation* (2013) **10**:46. doi:10.1186/1742-2094-10-46
287. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol* (2005) **57**:67–81. doi:10.1002/ana.20315
288. Uppal N, Gianatiempo I, Wicinski B, Schmeidler J, Heinsen H, Schmitz C, et al. Neuropathology of the posteroinferior occipitotemporal gyrus in children with autism. *Mol Autism* (2014) **5**(1):17. doi:10.1186/2040-2392-5-17
289. Jacot-Descombes S, Uppal N, Wicinski B, Santos M, Schmeidler J, Giannakopoulos P, et al. Decreased pyramidal neuron size in Brodmann areas 44 and 45 in patients with autism. *Acta Neuropathol* (2012) **124**:67–79. doi:10.1007/s00401-012-0976-6
290. Bauman ML, Kemper TL. Neuroanatomic observations of the brain in autism: a review and future directions. *Int J Dev Neurosci* (2005) **23**:183–7. doi:10.1016/j.ijdevneu.2004.09.006
291. Bauman ML, Kemper TL. Histoanatomic observations of the brain in early infantile autism. *Neurology* (1985) **35**:866–7. doi:10.1212/WNL.35.6.866
292. Casanova MF, El-Baz A, Vanbogaert E, Narahari P, Switala A. A topographic study of minicolumnar core width by lamina comparison between autistic subjects and controls: possible minicolumnar disruption due to an anatomical element in-common to multiple laminae. *Brain Pathol* (2010) **20**:451–8. doi:10.1111/j.1750-3639.2009.00319.x
293. Opris I, Casanova MF. Prefrontal cortical minicolumn: from executive control to disrupted cognitive processing. *Brain* (2014) **137**:1863–75. doi:10.1093/brain/awt359
294. Casanova MF, El-Baz AS, Kamat SS, Dombroski BA, Khalifa F, Elnakib A, et al. Focal cortical dysplasias in autism spectrum disorders. *Acta Neuropathol Commun* (2013) **1**:67. doi:10.1186/2051-5960-1-67
295. Alarcón M, Abraham BS, Stone JL, Duvall JA, Perederiy JV, Bomar JM, et al. Linkage, association, and gene expression analyses identify CNTNAP2 as an autism-susceptibility gene. *Am J Hum Genet* (2008) **82**:150–9. doi:10.1016/j.ajhg.2007.09.005
296. Piggot J, Shirinyan D, Shemmassian S, Vazirian S, Alarcón M. Neural systems approaches to the neurogenetics of autism spectrum disorders. *Neuroscience* (2009) **164**:247–56. doi:10.1016/j.neuroscience.2009.05.054
297. Kotagiri P, Chance SA, Szele FG, Esiri MM. Subventricular zone cytoarchitecture changes in autism. *Dev Neurobiol* (2014) **74**:25–41. doi:10.1002/dneu.22127
298. Gozzi M, Nielson DM, Lenroot RK, Ostuni JL, Luckenbaugh DA, Thurm AE, et al. A magnetization transfer imaging study of corpus callosum myelination in young children with autism. *Biol Psychiatry* (2012) **72**:215–20. doi:10.1016/j.biopsych.2012.01.026
299. Fatemi SH, Aldinger KA, Ashwood P, Bauman ML, Blaha CD, Blatt GJ, et al. Consensus paper: pathological role of the cerebellum in autism. *Cerebellum* (2012) **11**:777–807. doi:10.1007/s12311-012-0355-9
300. Ritvo ER, Freeman BJ, Scheibel AB, Duong T, Robinson H, Guthrie D, et al. Lower Purkinje cell counts in the cerebella of four autistic subjects: initial findings of the UCLA-NSAC autopsy research report. *Am J Psychiatry* (1986) **143**:862–6.
301. Gaffney GR, Tsai LY, Kuperman S, Minchin S. Cerebellar structure in autism. *Am J Dis Child* (1987) **141**:1330–2.
302. Whitney ER, Kemper TL, Bauman ML, Rosene DL, Blatt GJ. Cerebellar Purkinje cells are reduced in a subpopulation of autistic brains: a stereological experiment using calbindin-D28k. *Cerebellum* (2008) **7**:406–16. doi:10.1007/s12311-008-0043-y
303. Scott JA, Schumann CM, Goodlin-Jones BL, Amaral DG. A comprehensive volumetric analysis of the cerebellum in children and adolescents with autism spectrum disorder. *Autism Res* (2009) **2**:246–57. doi:10.1002/aur.97
304. Courchesne E, Saitoh O, Yeung-Courchesne R, Press GA, Lincoln AJ, Haas RH, et al. Abnormality of cerebellar vermian lobules VI and VII in patients with infantile autism: identification of hypoplastic and hyperplastic subgroups by MR imaging. *AJR Am J Roentgenol* (1994) **162**:123–30. doi:10.2214/ajr.162.1.8273650
305. Jeong JW, Tiwari VN, Behen ME, Chugani HT, Chugani DC. In vivo detection of reduced Purkinje cell fibers with diffusion MRI tractography in

children with autistic spectrum disorders. *Front Hum Neurosci* (2014) **8**:110. doi:10.3389/fnhum.2014.00110
306. Perry EK, Lee ML, Martin-Ruiz CM, Court JA, Volsen SG, Merrit J. Cholinergic activity in autism: abnormalities in the cerebral cortex and basal forebrain. *Am J Psychiatry* (2001) **158**:1058–66. doi:10.1176/appi.ajp.158.7.1058
307. Lee M, Martin-Ruiz C, Graham A, Court J, Jaros E, Perry R. Nicotinic receptor abnormalities in the cerebellar cortex in autism. *Brain* (2002) **125**:1483–95. doi:10.1093/brain/awf160
308. Blatt GJ, Fitzgerald CM, Guptill JT, Booker AB, Kemper TL, Bauman ML. Density and distribution of hippocampal neurotransmitter receptors in autism: an autoradiographic study. *J Autism Dev Disord* (2001) **31**:537–43. doi:10.1023/A:1013238809666
309. Oblak AL, Gibbs TT, Blatt GJ. Decreased GABA(B) receptors in the cingulate cortex and fusiform gyrus in autism. *J Neurochem* (2010) **114**:1414–23. doi:10.1111/j.1471-4159.2010.06858.x
310. Oblak AL, Gibbs TT, Blatt GJ. Reduced GABA(A) receptors and benzodiazepine binding sites in the posterior cingulate cortex and fusiform gyrus in autism. *Brain Res* (2011) **1380**:218–28. doi:10.1016/j.brainres.2010.09.021
311. Fatemi SH, Reutiman TJ, Folsom TD, Thuras PD. GABAA receptor downregulation in brains of subjects with autism. *J Autism Dev Disord* (2009) **39**:223–30. doi:10.1007/s10803-008-0646-7
312. Fatemi SH, Halt AR, Stary JM, Kanodia R, Schulz SC, Realmuto GR. Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autistic parietal and cerebellar cortices. *Biol Psychiatry* (2002) **52**:805–10. doi:10.1016/S0006-3223(02)01430-0
313. Fatemi SH, Folsom TD. Dysregulation of fragile x mental retardation protein and metabotropic glutamate receptor 5 in superior frontal cortex of individuals with autism: a postmortem brain study. *Mol Autism* (2011) **2**:6. doi:10.1186/2040-2392-2-6
314. Fatemi SH, Folsom TD, Kneeland RE, Liesch SB. Metabotropic glutamate receptor 5 up-regulation in children with autism is associated with underexpression of both fragile X mental retardation protein and GABAA receptor beta 3 in adults with autism. *Anat Rec* (2011) **294**:1635–45. doi:10.1002/ar.21299
315. Rustan OG, Folsom TD, Yousefi MK, Fatemi SH. Phosphorylated fragile X mental retardation protein at serine 499, is reduced in cerebellar vermis and superior frontal cortex of subjects with autism: implications for fragile X mental retardation protein-metabotropic glutamate receptor 5 signaling. *Mol Autism* (2013) **14**(1):41. doi:10.1186/2040-2392-4-41
316. Oblak A, Gibbs TT, Blatt GJ. Reduced serotonin receptor subtypes in a limbic and a neocortical region in autism. *Autism Res* (2013) **6**:571–83. doi:10.1002/aur.1317
317. Kang JQ, Barnes G. A common susceptibility factor of both autism and epilepsy: functional deficiency of GABA A receptors. *J Autism Dev Disord* (2013) **43**:68–79. doi:10.1007/s10803-012-1543-7
318. Betancur C, Buxbaum JD. SHANK3 haploinsufficiency: a "common" but underdiagnosed highly penetrant monogenic cause of autism spectrum disorders. *Mol Autism* (2013) **4**:17. doi:10.1186/2040-2392-4-17
319. Abdallah MW, Mortensen EL, Greaves-Lord K, Larsen N. Neonatal levels of neurotrophic factors and risk of autism spectrum disorders. *Acta Psychiatr Scand* (2013) **128**:61–9. doi:10.1111/acps.12020
320. Moessner R, Marshall CR, Sutcliffe JS, Skaug J, Pinto D, Vincent J, et al. Contribution of SHANK3 mutations to autism spectrum disorder. *Am J Hum Genet* (2007) **81**:1289–97. doi:10.1086/522590
321. Betancur C. Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting. *Brain Res* (2011) **1380**:42–77. doi:10.1016/j.brainres.2010.11.078
322. Chatonnet F, Flamant F, Morte B. A temporary compendium of thyroid hormone target genes in brain. *Biochim Biophys Acta* (2014). doi:10.1016/j.bbagrm.2014.05.023
323. Pescucci C, Meloni I, Bruttini M, Ariani F, Longo I, Mari F, et al. Chromosome 2 deletion encompassing the MAP2 gene in a patient with autism and Rett-like features. *Clin Genet* (2003) **64**:497–501. doi:10.1046/j.1399-0004.2003.00176.x
324. Kalkman HO. Potential opposite roles of the extracellular signal-regulated kinase (ERK) pathway in autism spectrum and bipolar disorders. *Neurosci Biobehav Rev* (2012) **36**:2206–13. doi:10.1016/j.neubiorev.2012.07.008
325. Waltes R, Duketis E, Knapp M, Anney RJ, Huguet G, Schlitt S, et al. Common variants in genes of the postsynaptic FMRP signalling pathway are risk factors for autism spectrum disorders. *Hum Genet* (2014) **133**:781–92. doi:10.1007/s00439-013-1416-y
326. Cao F, Yin A, Wen G, Sheikh AM, Tauqeer Z, Malik M, et al. Alteration of astrocytes and Wnt/β-catenin signaling in the frontal cortex of autistic subjects. *J Neuroinflammation* (2012) **9**(1):223. doi:10.1186/1742-2094-9-223
327. Chen YZ, Matsushita M, Girirajan S, Lisowski M, Sun E, Sul Y, et al. Evidence for involvement of GNB1L in autism. *Am J Med Genet B Neuropsychiatr Genet* (2012) **159B**:61–71. doi:10.1002/ajmg.b.32002
328. Emanuele E, Orsi P, Barale F, di Nemi SU, Bertona M, Politi P. Serum levels of vascular endothelial growth factor and its receptors in patients with severe autism. *Clin Biochem* (2010) **43**:317–9. doi:10.1016/j.clinbiochem.2009.10.005
329. Kim SJ, Gonen D, Hanna GL, Leventhal BL, Cook EH Jr. Deletion polymorphism in the coding region of the human NESP55 alternative transcript of GNAS1. *Mol Cell Probes* (2000) **14**:191–4. doi:10.1006/mcpr.2000.0300
330. Corbett BA, Kantor AB, Schulman H, Walker WL, Lit L, Ashwood P, et al. A proteomic study of serum from children with autism showing differential expression of apolipoproteins and complement proteins. *Mol Psychiatry* (2007) **12**:292–306. doi:10.1038/sj.mp.4001943
331. Garbett K, Ebert PJ, Mitchell A, Lintas C, Manzi B, Mirnics K, et al. Immune transcriptome alterations in the temporal cortex of subjects with autism. *Neurobiol Dis* (2008) **30**:303–11. doi:10.1016/j.nbd.2008.01.012
332. Stoner R, Chow ML, Boyle MP, Sunkin SM, Mouton PR, Roy S, et al. Patches of disorganization in the neocortex of children with autism. *N Engl J Med* (2014) **370**:1209–19. doi:10.1056/NEJMoa1307491
333. Pickett J, London E. The neuropathology of autism: a review. *J Neuropathol Exp Neurol* (2005) **64**:925–35. doi:10.1097/01.jnen.0000186921.42592.6c
334. Li J, Liu J, Zhao L, Ma Y, Jia M, Lu T, et al. Association study between genes in reelin signaling pathway and autism identifies DAB1 as a susceptibility gene in a Chinese Han population. *Prog Neuropsychopharmacol Biol Psychiatry* (2013) **44**:226–32. doi:10.1016/j.pnpbp.2013.01.004
335. Du X, An Y, Yu L, Liu R, Qin Y, Guo X, et al. A genomic copy number variant analysis implicates the MBD5 and HNRNPU genes in Chinese children with infantile spasms and expands the clinical spectrum of 2q23.1 deletion. *BMC Med Genet* (2014) **15**:62. doi:10.1186/1471-2350-15-62
336. Fatemi SH. Reelin glycoprotein: structure, biology and roles in health and disease. *Mol Psychiatry* (2005) **10**:251–7. doi:10.1038/sj.mp.4001613
337. Persico AM, D'Agruma L, Maiorano N, Totaro A, Militerni R, Bravaccio C, et al. Collaborative linkage study of autism. Reelin gene alleles and haplotypes as a factor predisposing to autistic disorder. *Mol Psychiatry* (2001) **6**:150–9. doi:10.1038/sj.mp.4000850
338. Zhang H, Liu X, Zhang C, Mundo E, Macciardi F, Grayson DR. Reelin gene alleles and susceptibility for autism spectrum disorders. *Mol Psychiatry* (2002) **7**:1012–7. doi:10.1038/sj.mp.4001124
339. Skaar DA, Shao Y, Haines JL, Stenger JE, Jaworski J, Martin ER, et al. Analysis of the RELN gene as a genetic risk factor for autism. *Mol Psychiatry* (2005) **10**:563–71. doi:10.1038/sj.mp.4001614
340. Serajee FJ, Zhong H, Mahbubul Huq AH. Association of reelin gene polymorphisms with autism. *Genomics* (2006) **87**:75–83. doi:10.1016/j.ygeno.2005.09.008
341. Krebs MO, Betancur C, Leroy S, Bourdel MC, Gillberg C, Leboyer M, et al. Absence of association between a polymorphic GGC repeat in the 50 untranslated region of the reelin gene and autism. *Mol Psychiatry* (2002) **7**:801–4. doi:10.1038/sj.mp.4001071
342. Bonora E, Beyer KS, Lamb JA, Parr JR, Klauck SM, Benner A, et al. International molecular genetic study of autism (IMGSAC). Analysis of reelin as a candidate gene for autism. *Mol Psychiatry* (2003) **10**:885–92. doi:10.1038/sj.mp.4001310
343. Devlin B, Bennett P, Dawson G, Figlewicz DA, Grigorenko EL, McMahon W, et al. Alleles of a reelin CGG repeat do not convey liability to autism in a sample from the CPEA network. *Am J Med Genet* (2004) **126B**:46–50. doi:10.1002/ajmg.b.20125
344. Li J, Nguyen L, Gleason C, Lotspeich L, Spiker D, Risch N. Lack of evidence for an association between WNT2 and RELN polymorphisms and autism. *Am J Med Genet* (2004) **126B**:51–7. doi:10.1002/ajmg.b.20122
345. Wang Z, Hong Y, Zou L, Zhong R, Zhu B, Shen N, et al. Reelin gene variants and risk of autism spectrum disorders: an integrated meta-analysis. *Am J Med Genet B Neuropsychiatr Genet* (2014) **165**:192–200. doi:10.1002/ajmg.b.32222
346. Bi C, Wu J, Jiang T, Liu Q, Cai W, Yu P, et al. Mutations of ANK3 identified by exome sequencing are associated with autism susceptibility. *Hum Mutat* (2012) **33**:1635–8. doi:10.1002/humu.22174

347. Turner G, Partington M, Kerr B, Mangelsdorf M, Gecz J. Variable expression of mental retardation, autism, seizures, and dystonic hand movements in two families with an identical ARX gene mutation. *Am J Med Genet* (2002) **112**:405–11. doi:10.1002/ajmg.10714
348. Egger G, Roetzer KM, Noor A, Lionel AC, Mahmood H, Schwarzbraun T, et al. Identification of risk genes for autism spectrum disorder through copy number variation analysis in Austrian families. *Neurogenetics* (2014) **15**:117–27. doi:10.1007/s10048-014-0394-0
349. Nishimura K, Nakamura K, Anitha A, Yamada K, Tsujii M, Iwayama Y, et al. Genetic analyses of the brain-derived neurotrophic factor (BDNF) gene in autism. *Biochem Biophys Res Commun* (2007) **356**:200–6. doi:10.1016/j.bbrc.2007.02.135
350. Zuko A, Kleijer KT, Oguro-Ando A, Kas MJ, van Daalen E, van der Zwaag B, et al. Contactins in the neurobiology of autism. *Eur J Pharmacol* (2013) **719**:63–74. doi:10.1016/j.ejphar.2013.07.016
351. Kim HW, Cho SC, Kim JW, Cho IH, Kim SA, Park M, et al. Family-based association study between NOS-I and -IIA polymorphisms and autism spectrum disorders in Korean trios. *Am J Med Genet B Neuropsychiatr Genet* (2009) **150B**:300–6. doi:10.1002/ajmg.b.30798
352. Yang W, Liu J, Zheng F, Jia M, Zhao L, Lu T, et al. The evidence for association of ATP2B2 polymorphisms with autism in Chinese Han population. *PLoS One* (2013) **8**(4):e61021. doi:10.1371/journal.pone.0061021
353. Morte B, Martínez de Arrieta C, Manzano J, Coloma A, Bernal J. Identification of a cis-acting element that interferes with thyroid hormone induction of the neurogranin (NRGN) gene. *FEBS Lett* (1999) **464**:179–83. doi:10.1016/S0014-5793(99)01706-8
354. Kelleher RJ III, Geigenmüller U, Hovhannisyan H, Trautman E, Pinard R, Rathmell B, et al. High-throughput sequencing of mGluR signaling pathway genes reveals enrichment of rare variants in autism. *PLoS One* (2012) **7**(4):e35003. doi:10.1371/journal.pone.0035003
355. Sicca F, Imbrici P, D'Adamo MC, Moro F, Bonatti F, Brovedani P, et al. Autism with seizures and intellectual disability: possible causative role of gain-of-function of the inwardly-rectifying K+ channel Kir4.1. *Neurobiol Dis* (2011) **43**:239–47. doi:10.1016/j.nbd.2011.03.016
356. Lassig JP, Vachirasomtoon K, Hartzell K, Leventhal M, Courchesne E, Courchesne R, et al. Physical mapping of the serotonin 5-HT(7) receptor gene (HTR7) to chromosome 10 and pseudogene (HTR7P) to chromosome 12, and testing of linkage disequilibrium between HTR7 and autistic disorder. *Am J Med Genet* (1999) **88**:472–5. doi:10.1002/(SICI)1096-8628(19991015)88:5<472::AID-AJMG7>3.0.CO;2-G
357. Gadad BS, Hewitson L, Young KA, German DC. Neuropathology and animal models of autism: genetic and environmental factors. *Autism Res Treat* (2013) **2013**:731935. doi:10.1155/2013/731935
358. Provenzano G, Zunino G, Genovesi S, Sgadó P, Bozzi Y. Mutant mouse models of autism spectrum disorders. *Dis Markers* (2012) **33**:225–39. doi:10.3233/DMA-2012-0917
359. Sadamatsu M, Kanai H, Xu X, Liu Y, Kato N. Review of animal models for autism: implication of thyroid hormone. *Congenit Anom (Kyoto)* (2006) **46**:1–9. doi:10.1111/j.1741-4520.2006.00094.x
360. Moy SS, Riddick NV, Nikolova VD, Teng BL, Agster KL, Nonneman RJ, et al. Repetitive behavior profile and supersensitivity to amphetamine in the C58/J mouse model of autism. *Behav Brain Res* (2014) **259**:200–14. doi:10.1016/j.bbr.2013.10.052
361. Sgadò P, Genovesi S, Kalinovsky A, Zunino G, Macchi F, Allegra M, et al. Loss of GABAergic neurons in the hippocampus and cerebral cortex of engrailed-2 null mutant mice: implications for autism spectrum disorders. *Exp Neurol* (2013) **247**:496–505. doi:10.1016/j.expneurol.2013.01.021
362. Sgadò P, Provenzano G, Dassi E, Adami V, Zunino G, Genovesi S, et al. Transcriptome profiling in engrailed-2 mutant mice reveals common molecular pathways associated with autism spectrum disorders. *Mol Autism* (2013) **4**(1):5. doi:10.1186/2040-2392-4-51
363. Burket JA, Benson AD, Tang AH, Deutsch SI. Rapamycin improves sociability in the BTBR T(+)Itpr3(tf)/J mouse model of autism spectrum disorders. *Brain Res Bull* (2014) **100**:70–5. doi:10.1016/j.brainresbull.2013.11.005
364. Ellegood J, Babineau BA, Henkelman RM, Lerch JP, Crawley JN. Neuroanatomical analysis of the BTBR mouse model of autism using magnetic resonance imaging and diffusion tensor imaging. *Neuroimage* (2012) **70**:288–300. doi:10.1016/j.neuroimage.2012.12.029
365. Uchino S, Waga C. SHANK3 as an autism spectrum disorder-associated gene. *Brain Dev* (2013) **35**:106–10. doi:10.1016/j.braindev.2012.05.013
366. Waga C, Asano H, Sanagi T, Suzuki E, Nakamura Y, Tsuchiya A, et al. Identification of two novel Shank3 transcripts in the developing mouse neocortex. *J Neurochem* (2014) **128**:280–93. doi:10.1111/jnc.12505
367. Almeida LE, Roby CD, Krueger BK. Increased BDNF expression in fetal brain in the valproic acid model of autism. *Mol Cell Neurosci* (2014) **59C**:57–62. doi:10.1016/j.mcn.2014.01.007
368. Martin HG, Manzoni OJ. Late onset deficits in synaptic plasticity in the valproic acid rat model of autism. *Front Cell Neurosci* (2014) **8**:23. doi:10.3389/fncel.2014.00023
369. Chomiak T, Hung J, Cihal A, Dhaliwal J, Baghdadwala M, Dzwonek A, et al. Auditory-cued sensorimotor task reveals disengagement deficits in rats exposed to the autism-associated teratogen valproic acid. *Neuroscience* (2014) **268**:212–20. doi:10.1016/j.neuroscience.2014.02.049
370. Chomiak T, Hu B. Alterations of neocortical development and maturation in autism: insight from valproic acid exposure and animal models of autism. *Neurotoxicol Teratol* (2013) **36**:57–66. doi:10.1016/j.ntt.2012.08.005
371. Engineer CT, Centanni TM, Im KW, Borland MS, Moreno NA, Carraway RS, et al. Degraded auditory processing in a rat model of autism limits the speech representation in non-primary auditory cortex. *Dev Neurobiol* (2014). doi:10.1002/dneu.22175
372. Sahu JK, Gulati S, Kabra M, Arya R, Sharma R, Gupta N, et al. Evaluation of subclinical hypothyroidism in ambulatory children with controlled epilepsy on valproate monotherapy. *J Child Neurol* (2012) **27**:594–7. doi:10.1177/0883073811421985
373. Armon C, Brown E, Carwile S, Miller P, Shin C. Sensorineural hearing loss: a reversible effect of valproic acid. *Neurology* (1990) **40**:1896–8. doi:10.1212/WNL.40.12.1896

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Transport of thyroid hormone in brain

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Thyroid hormone (TH) transport into the brain is not only pivotal for development and differentiation, but also for maintenance and regulation of adult central nervous system (CNS) function. In this review, we highlight some key factors and structures regulating TH uptake and distribution. Serum TH binding proteins play a major role for the availability of TH since only free hormone concentrations may dictate cellular uptake. One of these proteins, transthyretin is also present in the cerebrospinal fluid (CSF) after being secreted by the choroid plexus. Entry routes into the brain like the blood–brain-barrier (BBB) and the blood–CSF-barrier will be explicated regarding fetal and adult status. Recently identified TH transmembrane transporters (THTT) like monocarboxylate transporter 8 (Mct8) play a major role in uptake of TH across the BBB but as well in transport between cells like astrocytes and neurons within the brain. Species differences in transporter expression will be presented and interference of TH transport by endogenous and exogenous compounds including endocrine disruptors and drugs will be discussed.

Keywords: blood–brain-barrier, transthyretin, deiodinase, flavonoids, endocrine disruptors, Mct8, L-type amino acid transporter, organic anion transporters

MOLECULES INVOLVED IN TH TRANSPORT IN THE BRAIN

The hydrophobic but amphipathic charged amino acid-derived thyroid hormones (TH) are carried and distributed by several binding proteins from their site of production, storage, and secretion, the thyroid gland, to their target tissues including the brain. In human blood, four major proteins, thyroxine-binding globulin (TBG), transthyretin (TTR), albumin, and apolipoprotein B 100 (ApoB100), bind more than 99% of the circulating TH T_4, T_3, and 3-iodo-thyronamine (3-T1AM). In contrast, only TTR has been found as one of the main proteins in CSF where it is produced and directionally secreted by choroid plexus (CP) epithelial cells into the liquor, which does not contain the high affinity TBG or the high capacity albumin TH binding proteins. Whether 3-T1AM, a TH-derived biogenic amine and its high affinity binding protein ApoB100 occur in CSF, remains to be studied.

Thyroid hormone enters the brain either directly via the blood–brain barrier (BBB) or indirectly via the blood–CSF-barrier (B–CSF-B), with the BBB route as the major entry path for the prohormone T_4. T_4 is locally metabolized by selenoenzymes to either active T_3 via Type 2 deiodinase (Dio2) or inactivated by Type 3 deiodinase (Dio3), to yield reverse T_3 (rT_3). rT_3, devoid of T_3-like action, might be involved in developmental regulation of neuronal migration guided by astrocytes and glial cells (1). Dio2 is mostly expressed in astrocytes and tanycytes while Dio3 is mainly found in neurons. Whether Type 1 deiodinase (Dio1), catalyzing both 5′-deiodination (activation of T_4 to T_3) and 5-deiodination (inactivation of T_4 and T_3) is species-dependently expressed in brain remains controversial (2, 3). Dual entry paths of TH and cell type-specific expression of functional Dio enzymes in the brain raise the issues of (i) coordinated transport of active TH and TH metabolites between various brain cell types, (ii) organized communication between peripheral, thyroid-derived, and brain TH, and (iii) demands, supply, and disposal of TH precursors, metabolites, and active TH.

Adequate TH supply for the brain is of eminent importance during development but not less relevant in the differentiated adult organism with its changing hormonal requirement for metabolic and environmental adaptation.

Components controlling TH availability and action have been described in brain stem and progenitor cells (4) and TH receptor (TR) expression in the human brain has been demonstrated decades ago (5). Already during the first trimester human brain expresses various TH transporters (see **Table 1**), Dio enzymes, TR-isoforms, and isotypes in a development- and cell type-specific manner. Later in human pregnancy during weeks 17–20, endothelial cells and astrocytes organize the BBB [see Ref. (6)]. Endothelial cells express the TH transporter organic anion transporter polypeptide 1C1 (OATP1C1), which limits brain access of TH, especially T_4. At this time point fetal thyroid already starts producing TH, thus disconnecting the fetal TH responsive system from the maternal source of TH, but still depending on further adequate maternal iodide supply.

Facilitated uptake and release of TH by TH transmembrane transporters (THTT) is essential for their intracellular availability. TH have to cross multiple membranes in order to reach their nuclear and mitochondrial receptors. Especially, the entry of TH into the brain via the BBB and their subsequent distribution throughout all brain areas poses challenges in form of membranes of different cell types to be crossed. The complex interaction and communication between astrocytes and neurons, demonstrated for metabolic as well as synaptic processes, is in place regarding TH metabolism and distribution throughout the brain. Therefore, the specific spatio-temporal distribution of TH in different areas and cell types of the brain is required during embryonic development

Table 1 | Summary of expression profiles of thyroid hormone transmembrane transporters (THTT) in various cell types of the brain of several species.

Transporter	Species	Areas of the brain	References
Slc16a2 (Mct8)	Mouse	Protein: cortex, hippocampus, cerebellum, choroid plexus, hypothalamus, tanycytes, vessels	(7–9)
	Human	Protein: cortex, hippocampus, choroid plexus, hypothalamus, tanycytes	
		Widespread expression in fetal brain	(6–8, 10–12)
	Rat	Protein: hippocampus, tanycytes, vessels	(8, 11, 13)
	Chicken	Transcript: brain	(14, 15)
	Siberian hamster	Transcript: hypothalamus	(16)
	Rabbit	Transcript: hypothalamus	(17)
	Zebrafish	Transcript: brain	(18, 19)
	Fathead minnow	Transcript: cortex, cerebellum, hypothalamus	(20)
	Xenopus tropicalis	Transcript: brain	(21)
Slc16a10 (Mct10)	Mouse	Transcript: cortex, hippocampus, choroid plexus	(7, 9)
	Human	Protein: cortex, choroid plexus, hypothalamus	(7, 10, 22)
	Rabbit	Transcript: hypothalamus	(17)
	Fathead minnow	Transcript: cortex, cerebellum, hypothalamus	(20)
	Xenopus tropicalis	Transcript: brain	(21)
Slc7a5 (Lat1)	Mouse	Transcript: hippocampus, choroid plexus	(7, 9)
		Protein: cortex, cerebellum	
	Human	Transcript: cortex	(7, 12)
	Xenopus tropicalis	Transcript: brain	(21)
Slc7a8 (Lat2)	Mouse	Protein: cortex, hippocampus, cerebellum, choroid plexus	(7, 9)
	Human	Protein: adult: cortex, hippocampus, choroid plexus; fetal: microglia	(7, 12)
Slco1c1 (Oatp14)	Mouse	Transcript: cortex, hippocampus	(7–9, 23)
		Protein: choroid plexus, tanycytes, vessels	
	Human	Transcript: cortex	(7, 8, 10, 12, 22)
		Protein: choroid plexus, hypothalamus	
	Rat	Protein: choroid plexus, vessels	(8, 24)
	Chicken	Transcript: brain	(14, 15)
	Rabbit	Transcript: hypothalamus	(17)
	Fathead minnow	Transcript: cortex, cerebellum, hypothalamus	(20)
	Xenopus tropicalis	Transcript: brain	(21)

If available, protein data is preferably mentioned. Transcript data is only mentioned if no or only minimal protein data is available.

and differentiation, but also for adult maintenance and regulation of brain activity and metabolism.

ROLE OF TH BINDING AND DISTRIBUTOR PROTEINS FOR TH AVAILABILITY TO BRAIN CELLS

Tissue and cellular uptake depends on free TH concentrations in blood and both free T_4 and free T_3 are available for cellular uptake by THTT, while TH bound with high affinity to serum distributor proteins TBG and TTR is assumed not to be directly available for cellular uptake during organ perfusion by blood (25). In contrast, TH bound to albumin with high capacity but low affinity is easily liberated based on the high TH off-rate constants shown in liver perfusion (26, 27). While peripheral sensory neurons internalize TTR in megalin-dependent manner in context of neuritogenesis *in vitro* (28), evidence is missing, that TH–TTR ligand–protein complexes are taken up by neurons, astrocytes, or glial cells via receptors for these proteins expressed on brain cells. Such "trojan horse" mechanisms of ligand transmembrane transfer have been demonstrated during fetal development for several protein bound (pro-)hormones and vitamins such as TH, retinol, vitamin D3, and steroid hormones (29–31) for several peripheral target tissues including adult kidney but not yet for the fetal brain. Such mechanisms, mediated by megalin or cubilin receptors, might provide entry routes for hormones bypassing the concept of the "free hormone hypothesis" (32) and use of THTT.

Independent from such a mechanism, the high expression of evolutionary conserved TTR in CP and meninges of the developing and adult brain offers a further exchange compartment for TH, especially T_4, a high affinity ligand of TTR. During fetal brain development, the prominent voluminous CP is required for proper CNS growth and differentiation. TTR is the only TH binding protein expressed and directionally secreted into CSF. TTR, first described in CSF and subsequently in plasma, constitutes up to 20% of CSF protein and its secretion into CSF starts already in fetal week 8 before its hepatic production (28). Whether its adequate function as binding and distribution protein for two major morphogen precursors, T_4 and RBP-bound retinol, is essential or redundant, remains to be established. Lack of a major phenotype of TTR gene inactivation in the mouse came as a surprise with respect to normal brain development, HPT axis, TH homeostasis, and retinol-dependent functions (33). These mice grew normal, were fertile and had normal tissue T_4 levels though plasma TH concentrations were significantly reduced. Apparently, lack of TTR can be compensated by a shift of TH binding to rodent TBG, which has lower TH affinity compared to human TBG (34). TH metabolism and T_3-responsive gene expression of HPT axis and liver was unchanged in TTR knock-out mice. These observations either suggest a minor role for TTR-dependent TH binding and directional transport into CSF for proper brain development (35) or indicate existence of still unknown compensatory mechanisms active in absence of TTR during mouse development. At least this mouse model did not support the hypothesis that hormone binding and distribution proteins such as TTR in case of TH directly contribute to cellular hormone uptake as proposed by Pardridge (36) in distinction to several observations by the group of Willnow (31).

In contrast to these observations in the TTR knock-out mouse model are some findings on effects of endogenous or exogenous ligands of TTR, such as (iso-)flavonoids and endocrine disruptors, interfering with TH homeostasis in circulation, in CNS, and during fetal development. Several natural flavonoids, secondary metabolites of plants contained in our regular diet, avidly bind to TTR and displace TH from TTR binding based on their structural resemblance to TH. Resulting elevated free TH blood concentrations increase renal TH loss (transiently), elevate TH tissues levels, and enhance TH transfer via the placenta into fetal circulation including the fetal brain. *In vitro* as well as rodent animal studies provided evidence for interference of flavonoids and other TTR binding endocrine disruptors with CP-derived TTR-mediated TH transfer into CSF and the brain, resulting in disturbed homeostasis of brain TH levels and bioavailability (37).

More studies are needed analyzing (i) interference by natural and synthetic flavonoids with TH transport, (ii) action of endocrine disrupters such as the flame retardants polybrominated diphenylethers (PBDE) during neuronal stem cell development (38), or (iii) impact of ligands and pharmaceuticals, structurally related to TH (39) and interfering with THTT function (40). Recently, molecular actions and biological functions have been initially characterized for so far "neglected or minor" endogenous TH metabolites such as acetic acid- (Tetrac, Triac) or amine-derivatives (3-T1AM) of TH (41) and 3,5-T2, the latter abundantly present in the CNS (42). This raises the questions, (i) whether they are active players in TH-regulated brain function during development and in the adult organism, (ii) whether and how these metabolites are generated and transported in the brain, and (iii) how their mode of action interferes with classical TH action, which is mainly mediated via T_3-liganded TR. New modes of action may be envisaged for these TH metabolites at the plasma membrane, on cytosolic signaling cascades, or on other subcellular compartments of brain cells (43–45).

THTT AMONG SPECIES

Many transporters have been shown to transport TH. The most specific THTT is the monocarboxylate transporter 8 (Mct8; Slc16a2). Up to date, it is the only transporter with TH as the exclusive substrate. All other transporters out of the classes of monocarboxylate transporters (MCT), organic anion transporting polypeptides (OATP), and L-type amino acid transporters (LAT) also transport other substrates like amino acids. Most data about the presence and localization of THTT has been generated in mice and humans. The following table summarizes expression of the most researched THTT Mct8, Mct10, Lat1, Lat2, and Oatp1c1 in various vertebrate species (**Table 1**).

Research focus has been on the only THTT identified to cause a human disease so far, i.e., Mct8. MCT8 is widely expressed in the human fetal brain in several cell types (**Table 1**). Strong transcript and protein signals were observed in the cortical plate and subplate, as well as in ventricular and subventricular zones. Throughout fetal development CP epithelial cells and ependymal cells express high levels of MCT8 (6–8).

Comparably high MCT8 expression has been reported for monkey brain, which expresses OATP1C1 and LAT1 albeit at much lower levels (46). MCT8 mutations cause a severe syndrome of psychomotor retardation, the Allan–Herndon–Dudley syndrome (AHDS) (47–50). This syndrome also comprises endocrine

manifestations with high circulating T_3, low T_4, and normal to elevated TSH levels. The mouse model for *Mct8*-deficiency replicates the endocrine phenotype, but it does not mimic the psychomotor retardation of the human syndrome (7, 51, 52). Since AHDS is not comparable to the classical phenotype of congenital hypothyroidism or cretinism, it is of great importance to identify THTT in brain areas of human brains, as well as in the model organism used for analyzing TH transport to be able to understand phenotypic variations between these models. Other animal models apart from *Mct8*-deficient mice will be needed to evaluate the involvement of THTT in basic brain development. Recently, zebrafish has been evaluated for developmental effects of *Mct8*-deficiency. Significant species differences with respect to cell types, time point, dynamics, and regulation of THTT especially in the developing but also the adult brain have been reported for humans, monkey, chicken, rodent, fish, and amphibian brain (**Table 1**). For example, we demonstrated the expression of an additional transporter, Lat2, in mouse neurons during development, which is not expressed in human developing neurons (7). Research on double knock-out mice of Mct8 with either Mct10 or Oatp1c1 yielded valuable data on the interplay and possible compensation between these transporters (53, 54). Simultaneous deletion of *Mct8* and *Oatp1c1* lead to the ablation of both T_3 and T_4 transport across BBB. Symptoms of brain hypothyroidism were intensified underlining the importance of THTT for proper brain development and function. Further research analyzing the developmental expression of THTT and comparing effects of loss of function among different species will yield important information on the temporal effects of these transporters on brain development.

STRUCTURAL ASPECTS OF THTT

Thyroid hormone transport proteins MCT8, MCT10, OATP1C1, as well as LAT1 and LAT2 belong to different subfamilies within a huge protein superfamily of transport proteins, the major facilitator superfamily, MFS (55). General insights into the function of such transmembrane proteins can be derived from pathogenic mutations, e.g., in MCT8 from patients affected by AHDS (56).

Substrate recognition by transport proteins is fundamentally different from ligand binding in, e.g., nuclear receptors: while receptors are optimized for high affinity binding of their ligands, this would be detrimental for transporters, as these have to release their substrates easily. Most receptors have one binding site, while THTT should have at least two – one accessible from the exterior and one accessible form the interior of the cell. These two binding sites may overlap and differ only according to conformational changes associated with transport. The question is thus, how can a transporter achieve specificity and at the same time prevent tight binding?

With the exception of MCT8, all other THTT transport additional substrates, namely amino acids, bile acids, or conjugated steroids. It should therefore be of particular interest to compare how different protein families have adapted to transport TH and whether the substrate–protein interactions are similar or not. Experimental structures are not available for any of the THTT. Homology models based on experimental structures have been created for OATP1C1 (57), MCT8 (39), and LAT1 (58).

The homology model of rat Oatp1c1 was based on three high resolution crystal structures of bacterial transport proteins, lactose permease LacY, glycerol–phosphate transporter GlpT, and multidrug resistance protein EmrD. Authors achieved similar models with all templates and highlighted sequence identities between Oatp1c1 and templates below 10%. Conserved amino acids between Oatp1c1 and bacterial transporters were known to be functionally important in LacY and GlpT and may therefore not be involved in substrate specificity. While this work nicely shows that Oatp1c1 conforms to the overall structure of the bacterial transporters, there is no information on how specificity is established (57).

Chemical probes reactive with cysteines (*p*-chloromercury benzenesulfonate, pCMBS) or histidines (diethylpyrocarbonate, DEPC) were used to modify MCT8 and to test its activity afterward (59, 60). This approach suggested that Cys481, Cys497, and His192 may be close to the substrate translocation channel. Mutation to Ala of these critical amino acids rendered MCT8 resistant to pCMBS and DEPC.

We created a MCT8 homology model based on the inward-open conformation of GlpT and identified two charged amino acids within the transmembrane domains, Asp498 and Arg445, which are essential for transport (39). The homology model predicted a salt bridge between both residues. We suggested interaction of TH carboxyl and amino groups with these amino acids during transport, since TH analogs lacking the carboxyl or amino groups are not transported by MCT8 (39). The salt bridge was later independently confirmed by charge reversal mutants (61). MCT8 accepts only L-T_3, L-T_4, L-rT_3, and L-3,3′-T_2 as substrates (39). Based on the occurrence of a His–Arg clamp pinching T_3 in the T3 receptor β structure, we tested the hypothesis that a His–Arg pair spaced by about 15Å could serve the same purpose in MCT8 (62). Mutation of His192 (which may work together with Arg445 in an outward open conformation) clearly demonstrated His192 participation in substrate recognition (63). Interestingly, His192 corresponds to Gln88 in MCT10, a closely related homolog of MCT8 unable to transport T_4 (64). Mutation of His415 and Arg301, conserved in MCT8 and MCT10, affected transport kinetics as expected from substrate interactions (63). These findings corroborate the usefulness of the MCT8 homology model and suggest how the substrate is bound by MCT8 – at least in the inward-open conformation.

Recently, a LAT1 homology model was presented based on the crystal structure of bacterial agmatine antiporter AdiC (58). Iodotyrosines were identified *in silico* as LAT1 substrates and confirmed experimentally. Interestingly, while carboxy and amino groups are present in all LAT1 substrates, modeling suggests that these functions are sampled by the transporter by backbone polar contacts instead of side chain contacts as predicted in MCT8 (58, 63). Different transporters may have adapted different strategies to recognize iodinated TH substrates.

THTT IN CELL TYPES OF THE BRAIN

Analyses of brain regions provide important insight into THTT distribution. Immunohistochemical staining for Mct8 in mouse brains did not only show typical neuronal staining patterns, but also staining in astrocytes, CP, and tanycytes (7). However, most techniques like *in situ* hybridization or immunohistochemistry

do not allow for a real cell type-specific resolution and detection. Effects of TH uptake into neurons vs. astrocytes can not be dissected in complete brains. Primary cultures of mouse brain cells facilitate selection of a single cell type and the evaluation of effects of THTT deletion. Primary cultures of mouse neurons, astrocytes, and microglia can be used to detect THTT expression and functionality, as well as the reaction of specific cells on different conditions of TH access. Genetically engineered mouse lines can be used to create cell cultures originating from wildtype and transporter-deficient mice. Employing these cultures, we detected expression of Mct8, Lat1, and Lat2 transcripts and proteins in neurons and astrocytes, while Lat2 is additionally expressed in microglia (9, 65). Functional uptake studies in *Mct8*- and *Lat2*-deficient primary neuronal and astrocyte cell cultures demonstrated involvement of both transporters in T_3 and T_4 uptake into neurons and astrocytes (7, 9). The fraction of TH uptake mediated by transporters of the Mct, Lat, and Oatp groups can also be monitored by utilizing inhibitors of these transporters. Transport by Mct can be inhibited with bromosulphophtalein (BSP), while 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) is a Lat inhibitor and probenicid inhibits transport by Oatps. Uptake studies with these inhibitors gave very similar results on the involvement of these groups of transporters in TH uptake to genetic inactivation (7, 9).

Up to date, all concepts of interaction and cooperation between neurons and astrocytes imply astrocytes as providers of energy, communication and T_3 for neurons. However, it has been shown that neurons are generally able to carry out all functions needed for energy metabolism by themselves. It is therefore quite possible that neurons are able to convert T_4 to T_3 by expressing functional Dio2. Cell type-specific animal models and primary cell cultures are of great importance to study the interaction of neurons and astrocytes regarding transfer of TH metabolites and regulation of deiodinases independent of TH uptake at the BBB.

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REFERENCES

1. Farwell AP, Dubord-Tomasetti SA, Pietrzykowski AZ, Stachelek SJ, Leonard JL. Regulation of cerebellar neuronal migration and neurite outgrowth by thyroxine and 3,3′,5′-triiodothyronine. *Brain Res Dev Brain Res* (2005) **154**(1):121–35. doi:10.1016/j.devbrainres.2004.07.016
2. Visser TJ, Leonard JL, Kaplan MM, Larsen PR. Kinetic evidence suggesting two mechanisms for iodothyronine 5′-deiodination in rat cerebral cortex. *Proc Natl Acad Sci U S A* (1982) **79**(16):5080–4. doi:10.1073/pnas.79.16.5080
3. Verhoelst CH, Roelens SA, Darras VM. Role of spatiotemporal expression of iodothyronine deiodinase proteins in cerebellar cell organization. *Brain Res Bull* (2005) **67**(3):196–202. doi:10.1016/j.brainresbull.2005.06.030
4. Lemkine GF, Raj A, Alfama G, Turque N, Hassani Z, Alegria-Prevot O, et al. Adult neural stem cell cycling in vivo requires thyroid hormone and its alpha receptor. *FASEB J* (2005) **19**(7):863–5. doi:10.1096/fj.04-2916fje
5. Bernal J, Pekonen F. Ontogenesis of the nuclear 3,5,3′-triiodothyronine receptor in the human fetal brain. *Endocrinology* (1984) **114**(2):677–9. doi:10.1210/endo-114-2-677
6. Chan SY, Hancox LA, Martin-Santos A, Loubiere LS, Walter MN, Gonzalez AM, et al. MCT8 expression in human fetal cerebral cortex is reduced in severe intrauterine growth restriction. *J Endocrinol* (2014) **220**(2):85–95. doi:10.1530/JOE-13-0400
7. Wirth EK, Roth S, Blechschmidt C, Hölter SM, Becker L, Racz I, et al. 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**(30):9439–49. doi:10.1523/JNEUROSCI.6055-08.2009 Epub 2009/07/31,
8. Roberts LM, Woodford K, Zhou M, Black DS, Haggerty JE, Tate EH, et al. Expression of the thyroid hormone transporters monocarboxylate transporter-8 (SLC16A2) and organic ion transporter-14 (SLCO1C1) at the blood-brain barrier. *Endocrinology* (2008) **149**(12):6251–61. doi:10.1210/en.2008-0378
9. Braun D, Kinne A, Bräuer AU, Sapin R, Klein MO, Köhrle J, et al. Developmental and cell type-specific expression of thyroid hormone transporters in the mouse brain and in primary brain cells. *Glia* (2011) **59**(3):463–71. doi:10.1002/glia.21116 Epub 2011/01/26,
10. Friesema EC, Visser TJ, Borgers AJ, Kalsbeek A, Swaab DF, Fliers E, et al. Thyroid hormone transporters and deiodinases in the developing human hypothalamus. *Eur J Endocrinol* (2012) **167**(3):379–86. doi:10.1530/EJE-12-0177
11. Kallo I, Mohacsik P, Vida B, Zeold A, Bardoczi Z, Zavacki AM, et al. A novel pathway regulates thyroid hormone availability in rat and human hypothalamic neurosecretory neurons. *PLoS One* (2012) **7**(6):e37860. doi:10.1371/journal.pone.0037860
12. Chan SY, Martin-Santos A, Loubiere LS, Gonzalez AM, Stieger B, Logan A, et al. The expression of thyroid hormone transporters in the human fetal cerebral cortex during early development and in N-Tera-2 neurodifferentiation. *J Physiol* (2011) **589**(Pt 11):2827–45. doi:10.1113/jphysiol.2011.207290 Epub 2011/04/14,
13. Sharlin DS, Gilbert ME, Taylor MA, Ferguson DC, Zoeller RT. The nature of the compensatory response to low thyroid hormone in the developing brain. *J Neuroendocrinol* (2010) **22**(3):153–65. doi:10.1111/j.1365-2826.2009.01947.x Epub 2010/01/01,
14. Geysens S, Ferran JL, Van Herck SL, Tylzanowski P, Puelles L, Darras VM. Dynamic mRNA distribution pattern of thyroid hormone transporters and deiodinases during early embryonic chicken brain development. *Neuroscience* (2012) **221**:69–85. doi:10.1016/j.neuroscience.2012.06.057
15. Van Herck SL, Geysens S, Delbaere J, Tylzanowski P, Darras VM. Expression profile and thyroid hormone responsiveness of transporters and deiodinases in early embryonic chicken brain development. *Mol Cell Endocrinol* (2012) **349**(2):289–97. doi:10.1016/j.mce.2011.11.012
16. Herwig A, de Vries EM, Bolborea M, Wilson D, Mercer JG, Ebling FJ, et al. Hypothalamic ventricular ependymal thyroid hormone deiodinases are an important element of circannual timing in the Siberian hamster (*Phodopus sungorus*). *PLoS One* (2013) **8**(4):e62003. doi:10.1371/journal.pone.0062003
17. Mebis L, Debaveye Y, Ellger B, Derde S, Ververs EJ, Langouche L, et al. Changes in the central component of the hypothalamus-pituitary-thyroid axis in a rabbit model of prolonged critical illness. *Crit Care* (2009) **13**(5):R147. doi:10.1186/cc8043
18. Arjona FJ, de Vrieze E, Visser TJ, Flik G, Klaren PH. Identification and functional characterization of zebrafish solute carrier Slc16a2 (Mct8) as a thyroid hormone membrane transporter. *Endocrinology* (2011) **152**(12):5065–73. doi:10.1210/en.2011-1166
19. Vatine GD, Zada D, Lerer-Goldshtein T, Tovin A, Malkinson G, Yaniv K, et al. Zebrafish as a model for monocarboxyl transporter 8-deficiency. *J Biol Chem* (2013) **288**(1):169–80. doi:10.1074/jbc.M112.413831
20. Muzzio AM, Noyes PD, Stapleton HM, Lema SC. Tissue distribution and thyroid hormone effects on mRNA abundance for membrane transporters Mct8, Mct10, and organic anion-transporting polypeptides (Oatps) in a teleost fish. *Comp Biochem Physiol A Mol Integr Physiol* (2014) **167**:77–89. doi:10.1016/j.cbpa.2013.09.019
21. Connors KA, Korte JJ, Anderson GW, Degitz SJ. Characterization of thyroid hormone transporter expression during tissue-specific metamorphic events in *Xenopus tropicalis*. *Gen Comp Endocrinol* (2010) **168**(1):149–59. doi:10.1016/j.ygcen.2010.04.015 Epub 2010/04/27,
22. Alkemade A, Friesema EC, Kalsbeek A, Swaab DF, Visser TJ, Fliers E. Expression of thyroid hormone transporters in the human hypothalamus. *J Clin Endocrinol Metab* (2011) **96**(6):E967–71. doi:10.1210/jc.2010-2750
23. Tohyama K, Kusuhara H, Sugiyama Y. Involvement of multispecific organic anion transporter, Oatp14 (Slc21a14), in the transport of thyroxine across the blood-brain barrier. *Endocrinology* (2004) **145**(9):4384–91. doi:10.1210/en.2004-0058

24. Sugiyama D, Kusuhara H, Taniguchi H, Ishikawa S, Nozaki Y, Aburatani H, et al. Functional characterization of rat brain-specific organic anion transporter (Oatp14) at the blood-brain barrier: high affinity transporter for thyroxine. *J Biol Chem* (2003) **278**(44):43489–95. doi:10.1074/jbc.M306933200
25. Richardson SJ. Evolutionary changes to transthyretin: evolution of transthyretin biosynthesis. *FEBS J* (2009) **276**(19):5342–56. doi:10.1111/j.1742-4658.2009.07244.x
26. Mendel CM, Weisiger RA, Jones AL, Cavalieri RR. Thyroid hormone-binding proteins in plasma facilitate uniform distribution of thyroxine within tissues: a perfused rat liver study. *Endocrinology* (1987) **120**(5):1742–9. doi:10.1210/endo-120-5-1742
27. Richardson SJ. Cell and molecular biology of transthyretin and thyroid hormones. *Int Rev Cytol* (2007) **258**:137–93. doi:10.1016/S0074-7696(07)58003-4
28. Fleming CE, Mar FM, Franquinho F, Saraiva MJ, Sousa MM. Transthyretin internalization by sensory neurons is megalin mediated and necessary for its neuritogenic activity. *J Neurosci* (2009) **29**(10):3220–32. doi:10.1523/JNEUROSCI.6012-08.2009
29. Sousa MM, Norden AG, Jacobsen C, Willnow TE, Christensen EI, Thakker RV, et al. Evidence for the role of megalin in renal uptake of transthyretin. *J Biol Chem* (2000) **275**(49):38176–81. doi:10.1074/jbc.M002886200
30. Willnow TE, Hammes A, Nykjaer A. Endocytosis of sex steroids: the hypothesis of free hormones revisited. *Ann Endocrinol (Paris)* (2008) **69**(2):101–2. doi:10.1016/j.ando.2008.02.024
31. Willnow TE, Nykjaer A. Cellular uptake of steroid carrier proteins – mechanisms and implications. *Mol Cell Endocrinol* (2010) **316**(1):93–102. doi:10.1016/j.mce.2009.07.021
32. Mendel CM. The free hormone hypothesis. Distinction from the free hormone transport hypothesis. *J Androl* (1992) **13**(2):107–16.
33. Episkopou V, Maeda S, Nishiguchi S, Shimada K, Gaitanaris GA, Gottesman ME, et al. Disruption of the transthyretin gene results in mice with depressed levels of plasma retinol and thyroid hormone. *Proc Natl Acad Sci U S A* (1993) **90**(6):2375–9. doi:10.1073/pnas.90.6.2375
34. Vranckx R, Savu L, Maya M, Nunez EA. Characterization of a major development-regulated serum thyroxine-binding globulin in the euthyroid mouse. *Biochem J* (1990) **271**(2):373–9.
35. Palha JA. Transthyretin as a thyroid hormone carrier: function revisited. *Clin Chem Lab Med* (2002) **40**(12):1292–300. doi:10.1515/CCLM.2002.223
36. Pardridge WM. Plasma protein-mediated transport of steroid and thyroid hormones. *Am J Physiol* (1987) **252**(2 Pt 1):E157–64.
37. Schröder-van der Elst JP, van der Heide D, Rokos H, Morreale de Escobar G, Köhrle J. Synthetic flavonoids cross the placenta in the rat and are found in fetal brain. *Am J Physiol* (1998) **274**(Pt 1):E253–6.
38. Morvan-Dubois G, Fini JB, Demeneix BA. Is thyroid hormone signaling relevant for vertebrate embryogenesis? *Curr Top Dev Biol* (2013) **103**:365–96. doi:10.1016/B978-0-12-385979-2.00013-7
39. Kinne A, Kleinau G, Hoefig CS, Grüters A, Köhrle J, Krause G, et al. Essential molecular determinants for thyroid hormone transport and first structural implications for monocarboxylate transporter 8. *J Biol Chem* (2010) **285**(36):28054–63. doi:10.1074/jbc.M110.129577
40. Braun D, Kim TD, le Coutre P, Kohrle J, Hershman JM, Schweizer U. Tyrosine kinase inhibitors noncompetitively inhibit MCT8-mediated iodothyronine transport. *J Clin Endocrinol Metab* (2012) **97**(1):E100–5. doi:10.1210/jc.2011-1837
41. Brix K, Fuhrer D, Biebermann H. Molecules important for thyroid hormone synthesis and action – known facts and future perspectives. *Thyroid Res* (2011) **4**(Suppl 1):S9. doi:10.1186/1756-6614-4-S1-S9 Epub 2011/08/13,
42. Pinna G, Meinhold H, Hiedra L, Thoma R, Hoell T, Graf KJ, et al. Elevated 3,5-diiodothyronine concentrations in the sera of patients with nonthyroidal illnesses and brain tumors. *J Clin Endocrinol Metab* (1997) **82**(5):1535–42. doi:10.1210/jcem.82.5.3939
43. Moeller LC, Broecker-Preuss M. Transcriptional regulation by nonclassical action of thyroid hormone. *Thyroid Res* (2011) **4**(Suppl 1):S6. doi:10.1186/1756-6614-4-S1-S6 Epub 2011/08/13,
44. Davis PJ, Lin HY, Tang HY, Davis FB, Mousa SA. Adjunctive input to the nuclear thyroid hormone receptor from the cell surface receptor for the hormone. *Thyroid* (2013) **23**(12):1503–9. doi:10.1089/thy.2013.0280
45. Brent GA. Mechanisms of thyroid hormone action. *J Clin Invest* (2012) **122**(9):3035–43. doi:10.1172/JCI60047
46. Ito K, Uchida Y, Ohtsuki S, Aizawa S, Kawakami H, Katsukura Y, et al. Quantitative membrane protein expression at the blood-brain barrier of adult and younger cynomolgus monkeys. *J Pharm Sci* (2011) **100**(9):3939–50. doi:10.1002/jps.22487 Epub 2011/01/22,
47. Schwartz CE, May MM, Carpenter NJ, Rogers RC, Martin J, Bialer MG, et al. Allan-Herndon-Dudley syndrome and the monocarboxylate transporter 8 (MCT8) gene. *Am J Hum Genet* (2005) **77**(1):41–53. doi:10.1086/431313
48. Biebermann H, Ambrugger P, Tarnow P, von Moers A, Schweizer U, Grueters A. Extended clinical phenotype, endocrine investigations and functional studies of a loss-of-function mutation A150V in the thyroid hormone specific transporter MCT8. *Eur J Endocrinol* (2005) **153**(3):359–66. doi:10.1530/eje.1.01980
49. 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**(1):168–75. doi:10.1086/380999
50. Friesema EC, Grueters A, Biebermann H, Krude H, von Moers A, Reeser M, et al. Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet* (2004) **364**(9443):1435–7. doi:10.1016/S0140-6736(04)17226-7
51. 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**(9):4036–43. doi:10.1210/en.2006-0390
52. Trajkovic M, Visser TJ, Mittag J, Horn S, Lukas J, Darras VM, et al. Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8. *J Clin Invest* (2007) **117**(3):627–35. doi:10.1172/JCI28253
53. Müller J, Mayerl S, Visser TJ, Darras VM, Boelen A, Frappart L, et al. Tissue-specific alterations in thyroid hormone homeostasis in combined Mct10 and Mct8 deficiency. *Endocrinology* (2014) **155**(1):315–25. doi:10.1210/en.2013-1800
54. Mayerl S, Müller J, Bauer R, Richert S, Kassmann CM, Darras VM, et al. Transporters MCT8 and OATP1C1 maintain murine brain thyroid hormone homeostasis. *J Clin Invest* (2014) **124**(5):1987–99. doi:10.1172/JCI70324
55. Kinne A, Schulein R, Krause G. Primary and secondary thyroid hormone transporters. *Thyroid Res* (2011) **4**(Suppl 1):S7. doi:10.1186/1756-6614-4-S1-S7 Epub 2011/08/13,
56. Kinne A, Roth S, Biebermann H, Köhrle J, Grüters A, Schweizer U. Surface translocation and tri-iodothyronine uptake of mutant MCT8 proteins are cell type-dependent. *J Mol Endocrinol* (2009) **43**(6):263–71. doi:10.1677/JME-09-0043
57. Westholm DE, Marold JD, Viken KJ, Duerst AH, Anderson GW, Rumbley JN. Evidence of evolutionary conservation of function between the thyroxine transporter Oatp1c1 and major facilitator superfamily members. *Endocrinology* (2010) **151**(12):5941–51. doi:10.1210/en.2010-0640
58. Geier EG, Schlessinger A, Fan H, Gable JE, Irwin JJ, Sali A, et al. Structure-based ligand discovery for the Large-neutral Amino Acid Transporter 1, LAT-1. *Proc Natl Acad Sci U S A* (2013) **110**(14):5480–5. doi:10.1073/pnas.1218165110
59. Lima de Souza EC, Groeneweg S, Visser WE, Peeters RP, Visser TJ. Importance of cysteine residues in the thyroid hormone transporter MCT8. *Endocrinology* (2013) **154**(5):1948–55. doi:10.1210/en.2012-2101
60. Groeneweg S, Lima de Souza EC, Visser WE, Peeters RP, Visser TJ. Importance of His192 in the human thyroid hormone transporter MCT8 for substrate recognition. *Endocrinology* (2013) **154**(7):2525–32. doi:10.1210/en.2012-2225
61. Groeneweg S, Friesema EC, Kersseboom S, Klootwijk W, Visser WE, Peeters RP, et al. The role of Arg445 and Asp498 in the human thyroid hormone transporter MCT8. *Endocrinology* (2014) **155**(2):618–26. doi:10.1210/en.2013-1521
62. Kleinau G, Schweizer U, Kinne A, Köhrle J, Grüters A, Krude H, et al. Insights into molecular properties of the human monocarboxylate transporter 8 by combining functional with structural information. *Thyroid Res* (2011) **4**(Suppl 1):S4. doi:10.1186/1756-6614-4-S1-S4 Epub 2011/08/13,
63. Braun D, Lelios I, Krause G, Schweizer U. Histidines in potential substrate recognition sites affect thyroid hormone transport by monocarboxylate transporter 8 (MCT8). *Endocrinology* (2013) **154**(7):2553–61. doi:10.1210/en.2012-2197
64. 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**(6):1357–69. doi:10.1210/me.2007-0112

65. Braun D, Wirth EK, Schweizer U. Thyroid hormone transporters in the brain. *Rev Neurosci* (2010) **21**(3):173–86. doi:10.1515/REVNEURO.2010.21.3.173 Epub 2010/10/01,

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Expression pattern of thyroid hormone transporters in the postnatal mouse brain

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For a comprehensive description of the tissue-specific thyroidal state under normal as well as under pathophysiological conditions it is of utmost importance to include thyroid hormone (TH) transporters in the analysis as well. The current knowledge of the cell-specific repertoire of TH transporters, however, is still rather limited, although several TH transporting proteins have been identified. Here, we describe the temporal and spatial distribution pattern of the most prominent TH transporters in the postnatal mouse brain. For that purpose, we performed radioactive *in situ* hybridization studies in order to analyze the cellular mRNA expression pattern of the monocarboxylate transporters Mct8 and Mct10, the L-type amino acid transporters Lat1 and Lat2 as well as the organic anion transporting peptide Oatp1c1 at different postnatal time points. Highest TH transporter expression levels in the CNS were observed at postnatal day 6 and 12, while hybridization signal intensities visibly declined after the second postnatal week. The only exception was Mct10 for which the strongest signals could be observed in white matter regions at postnatal day 21 indicating that this transporter is preferentially expressed in mature oligodendrocytes. Whereas Mct8 and Lat2 showed an overlapping neuronal mRNA expression pattern in the cerebral cortex, hippocampus, and in the hypothalamus, Oatp1c1 and Lat1 specific signals were most prominent in capillary endothelial cells throughout the CNS. In the choroid plexus, expression of three transporters (Mct8, Lat2, and Oatp1c1) could be detected, whereas in other brain areas (e.g., striatum, thalamus, and brain stem nuclei) only one of the transporter candidates appeared to be present. Overall, our study revealed a distinct mRNA distribution pattern for each of the TH transporter candidates. Further studies will reveal to which extent these transporters contribute to the cell-specific TH uptake and efflux in the mouse CNS.

Keywords: Mct8, Mct10, Oatp1c1, Lat1, Lat2, T3, T4, CNS

INTRODUCTION

Thyroid hormone (TH) action requires the presence of TH transporters that facilitate its cellular uptake and efflux (1–4). Since TH metabolizing deiodinases as well as TH receptors are intracellularly active, TH transporter deficiency can greatly compromise tissue TH homeostasis. As the most prominent example, inactivating mutations in the monocarboxylate transporter 8 (MCT8; SCL16A2) gene, which encodes a very specific TH transporter (5), result in an abnormal serum TH profile with highly elevated levels of the receptor active hormone T3 (3,3′,5,-triiodothyronine) and low T4 concentrations of the prohormone T4 (3,3′,5,5′-tetraiodothyronine) (6–8). Moreover, patients with MCT8 mutations suffer from a severe form of psychomotor retardation suggesting that in the absence of MCT8, neural differentiation and function is severely impaired possibly due to insufficient TH supply during critical stages of development (9). The exact role of MCT8 is still enigmatic since only limited information is currently available concerning the cellular localization of MCT8 in the developing and adult human CNS (10–12).

Studies of Mct8 knockout (ko) mice unequivocally revealed that Mct8 is not the only protein involved in TH transport processes in the murine CNS (11, 13, 14). Although immunohistochemical studies and *in situ* hybridization (ISH) experiments showed pronounced Mct8 expression in distinct neuronal populations of the cerebral cortex, hippocampus, striatum, hypothalamus, and cerebellum (15), Mct8 ko mice do not display overt neurological symptoms (11), although they faithfully replicate the abnormal serum TH parameters characteristic for human MCT8 deficiency. These Mct8 ko mice also do not show immunohistochemically any abnormalities such as a delayed Purkinje cell development or an altered differentiation of inhibitory neurons in the cerebral cortex, both strong neuronal indicators for TH deprivation (11, 14). Based on these observations, it was hypothesized that other TH transporting proteins can compensate for the absence of Mct8 in the mouse CNS. Indeed, analysis of primary neuronal cultures from the mouse cortex revealed a collaborative action of Mct8 and the L-type amino acid transporter Lat2 that in addition to large neutral amino acids also accepts TH as substrates (11). Which

transporters, however, are co-expressed with Mct8 *in vivo* has not been sufficiently addressed yet.

Apart from neurons, Mct8 is present in capillary endothelial cells as well as in the choroid plexus structures, thus in cells that build up the blood–brain (BBB) and the blood–cerebrospinal fluid-barrier (BCSFB), respectively (10, 11, 15). That Mct8 is indeed involved in the passage of TH via the BBB and/or BCSFB could be demonstrated by *in vivo* transport studies. Uptake of T3 from the circulation into the CNS was strongly diminished in Mct8 ko mice, whereas the transport of T4 was only mildly compromised (14, 16) due to the presence of the T4 transporting organic anion transporting peptide Oatp1c1 (Slco1c1) (17–19). Indeed, the generation and analysis of Mct8/Oatp1c1 double knockout (dko) mice confirmed the physiological significance of both TH transporters for proper TH homeostasis in the murine brain since the brain T3 and T4 content of these animals was reduced to 10% of wild-type levels (20). Obviously, Mct8 and Oatp1c1 act as a pair in mediating TH access to the CNS, although the residual TH amounts found in brain homogenates of Mct8/Oatp1c1 dko mice suggest that additional, not yet identified TH transporters contribute to this process as well.

From all these mouse studies it became strikingly clear that a detailed determination of the tissue- and cell-specific repertoire of TH transporters is highly needed for a comprehensive understanding of TH trafficking, metabolism, and action under normal as well as pathological conditions. Recently, Braun et al. generated a developmental profile of TH transporter expression patterns in different brain regions by performing western blot analysis and qPCR studies (21). However, these studies did not provide any information regarding the cellular localization pattern. We therefore conducted a series of ISH experiments that allow the temporal and spatial analysis of TH transporter expression with a cellular resolution in the postnatal mouse brain. In addition to Mct8, Oatp1c1, and Lat2, we included the aromatic amino acid transporter Mct10 (Slc16a10) and the L-type amino acid transporter Lat1 (Slc7a5) in our study since both proteins have been shown to accept TH as substrates (22, 23) and may play an important role for TH transmembrane passage in the CNS as well.

MATERIALS AND METHODS

ANIMALS

All mice were provided with standard laboratory chow and tap water *ad libitum* and were kept at constant temperature (22°C) and controlled light cycle (12 h light, 12 h dark). Male wild-type mice were killed in accordance with local regulations (TLLV Thüringen, Erfurt, Germany; approval number TöA-FLI149-08) by CO_2 at different postnatal time points (P6, P12, P21, P84) and brains were frozen in isopentane cooled on dry ice. For each time point, three brains were prepared. Coronal cryosections with a thickness of 20 µm were cut with a cryostat and thaw mounted on superfrost slides (Thermo Scientific). Slides were stored at −80°C until further processing.

IN SITU HYBRIDIZATION HISTOCHEMISTRY

A cDNA fragment corresponding to nt 1251–1876 of mouse Mct8 (GenBank accession number AF045692), nt 911–1663 of mouse Mct10 (NM_001114332), nt 921–1357 of mouse Lat1 (NM_011404.3), nt 972–1457 of mouse Lat2 (NM-016972.2), nt 360–470 of mouse Oatp1c1 (NM_021471.1) were generated by PCR and subcloned into the pGEM-T Easy Vector (Promega). Radiolabeled riboprobes were generated by *in vitro* transcription using ^{35}S-UTP as labeled substrate (Hartmann Analytik, Braunschweig, Germany). ISH was carried out as published elsewhere (24). In brief, frozen sections were air-dried, followed by an 1-h fixation in a 4% phosphate-buffered paraformaldehyde (PFA) solution (pH 7.4) and then permeabilized by incubation in 0.4% Triton-X 100/PBS for 10 min. Acetylation was carried out in 0.1 M triethanolamine (pH 8.0) containing 0.25% (v/v) acetic anhydride. Sections were dehydrated and then covered with hybridization mix containing cRNA probes diluted in hybridization buffer (50% formamide, 10% dextran sulfate, 0.6 M NaCl, 10 mM Tris–HCl pH 7.5, 1× Denhardt's solution, 100 µg/ml sonicated salmon sperm DNA, 1 mM EDTA, and 0.5 mg/ml t-RNA). 35S-labeled riboprobes were diluted in hybridization buffer to a final concentration of 1×10^4 cpm/µl (Mct8) or 2×10^4 cpm/µl (Lat1, Lat2, Oatp1c1). Hybridization was performed over night at 58°C. Slides were rinsed in 2× standard saline citrate (0.3 M NaCl and 0.03 M sodium citrate, pH 7.0) and subsequently treated with ribonuclease A/T1 at 37°C for 30 min. Final washes were carried out in 0.2× standard saline citrate at 65°C for 1 h. For detecting radioactive hybridization signals, the sections were dehydrated and then exposed to x-ray film (BioMax MR, Eastman Kodak Co.) for 24–48 h. Thereafter, sections were dipped in Kodak NTB nuclear emulsion (Kodak) and stored at 4°C for 8 days (Mct8), 7 days (Lat1), 6 days (Lat2), or 11 days (Oatp1c1). Autoradiograms were developed and analyzed under dark-field illumination. As controls, consecutive brain sections were probed with the sense-strand probes of the same size and specific activity and processed in the same manner as the sections covered with the respective antisense probe. For none of these sense probes has a positive signal been encountered.

RESULTS

The aim of the current study was to perform a comparative analysis of TH transporter expression patterns in the murine CNS at different postnatal time point. In particular, we aimed to determine the cellular distribution pattern of the transporters Mct8 (Slc16a2), Mct10 (Slc16a10), Lat1 (Slc7a5), Lat2 (Slc7a8), and Oatp1c1 (Slco1c1) that have all been described to facilitate the cellular transport of TH (25). Since only for a subset of these proteins specific antibodies are available, we employed a highly sensitive ISH technique using ^{35}S-labeled RNA probes. Brains were collected from C57/Bl6 male mice at postnatal day 6 (P6), P12, P21, and P84 and consecutive frozen brain sections were subjected to the ISH procedure as described previously (24). For the description of the mRNA distribution at a cellular resolution, nuclear emulsion coated slides were examined under dark-field illumination using a light-microscope. Each antisense probe indeed produced a positive hybridization signal with a highly distinct cellular distribution pattern as visualized exemplarily in **Figures 1–4** for four different anatomical regions. In particular, **Figure 1** illustrates cortical and striatal expression at around Bregma 0.50 mm; **Figure 2** (between Bregma −1.8 and −2.2 mm) shows mRNA expression patterns in the cortex, hippocampus, thalamus, and hypothalamus;

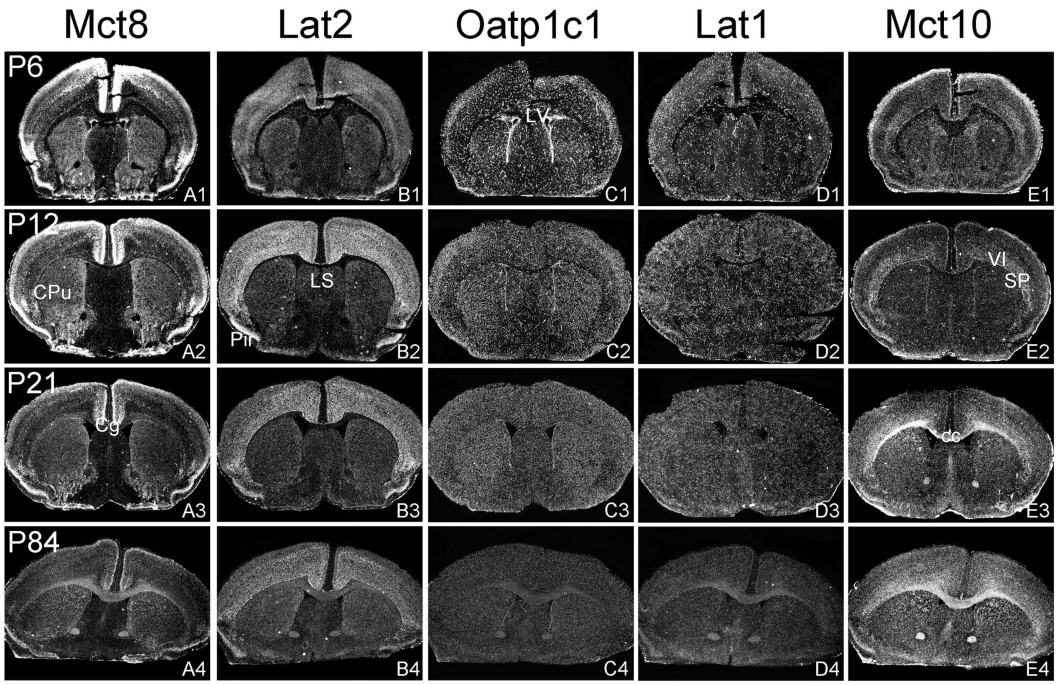

FIGURE 1 | Illustration of TH transporter mRNA expression in the murine forebrain. Coronal cryosections of mouse brains collected at different postnatal days were hybridized with radioactively labeled riboprobes specific for Mct8 **(A1–A4)**, Lat2 **(B1–B4)**, Oatp1c1 **(C1–C4)**, Lat1 **(D1–D4)**, and Mct10 **(E1–E4)**. Dark-field autoradiograms illustrate the mRNA expression patterns at the level of the striatum (CPu, caudate–putamen) around Bregma 0.5 mm. VI, layer 6 of the cerebral cortex; cc, corpus callosum; Cg, cingulate cortex, LV, lateral ventricle; Pir, piriform cortex; SP, subplate neurons (cerebral cortex).

Figure 3 depicts TH transporter expression between Bregma −3.4 and −4.4 including mesencephalic areas; **Figure 4** shows cerebellar and brainstem expression pattern around Bregma −6.2 [according to Franklin and Paxinos (26)].

DISTRIBUTION PATTERN OF Mct8 AND Lat2

In agreement with our previous analysis (15) and immunohistochemical studies (11), Mct8 was found in different brain areas with strongest expression levels in pyramidal and granule cells of the hippocampus, in the choroid plexus and in tanycytes of the third ventricle (**Figures 2A1–A4**). Pronounced hybridization signals were also found in the upper layers of the cerebral cortex, throughout the striatum (**Figures 1A1–A4**) as well as in cerebellar Purkinje cells particularly during the first postnatal weeks (**Figures 4A1–A4**). In these areas, Mct8 signal intensities were visibly reduced in the adult animals indicating a temporal decline in expression. A similar trend was also observed for Lat2 that exhibited an overlapping expression pattern with Mct8 in distinct areas such as the choroid plexus, the hippocampus, the cerebral cortex, and the hypothalamic neurons of the PVN (**Figures 2B1–B4**). Highest mRNA expression of Lat2, however, was observed throughout the thalamic region, an area devoid of Mct8 specific hybridization signals (**Figures 2B1–B4**). Lat2 specific mRNA signals were also found in the pontine nucleus (**Figures 3B1–B4**), various brain stem nuclei such as the facial nucleus, hypoglossal nucleus, and the raphe nuclei (**Figures 4B1–B4**) where Mct8 expression could not be detected. Another example for a complementary expression of Mct8 and Lat2 was observed in the cerebellar cortex where Mct8 transcript levels could be detected in granule precursor cells of the external granule cell layer at P6 as well as in developing Purkinje cells (at P6 and P12), whereas Lat2 was most intensely expressed in mature granule cells of the internal granule cell layer (**Figures 4A1–A4,B1–B4**). Overall, our results indicate a co-expression of both TH transporters only in distinct areas of the mouse CNS.

DISTRIBUTION PATTERN OF Oatp1c1 AND Lat1

Our ISH analysis confirmed published studies indicating a preferential expression of Oatp1c1 in choroid plexus structures, ventricle ependymal cells, and capillary endothelial cells (**Figures 1C1–C4** and **2C1–C4**) (10, 18, 27). In addition to the elongated hybridization signals typically for vessel structures, ISH analysis for Oatp1c1 also revealed scattered signals throughout the CNS with slightly stronger intensities in the hippocampal formation (**Figures 2C1–C4** and **3C1–C4**). Such an expression pattern points to an astroglial localization of this transporter in line with previous findings (28). A capillary localization could also be confirmed for Lat1. However, in contrast to Oatp1c1, Lat1 is not expressed in the choroid plexus. Moreover, at P6 and, to a lesser extent at P12, Lat1 specific hybridization signals were detected in few neuronal populations such as in pyramidal neurons of the hippocampal CA3 region and the cerebellar granule cells indicating that during early stages of postnatal development, Lat1 is also present in distinct neurons (**Figures 2D1–D4** and **4D1–D4**). For both transporters Oatp1c1 and Lat1, hybridization signal intensities were

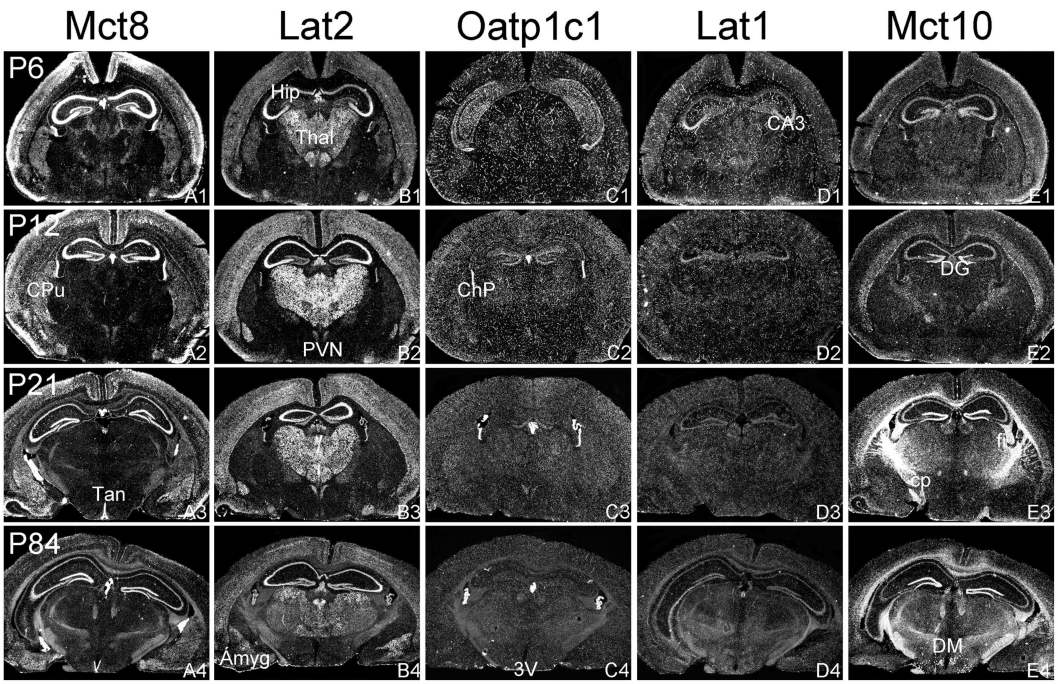

FIGURE 2 | mRNA distribution patterns of TH transporters in the murine hippocampus and diencephalon. Mct8 **(A1–A4)** and Lat2 **(B1–B4)** exhibited an overlapping mRNA expression in the cerebral cortex, hippocampus (Hip), amygdala (Amyg), hypothalamic paraventricular nucleus (PVN), and choroid plexus (ChP). Mct8 but not Lat2 is highly expressed in tanycytes (Tan) lining the third ventricle (3V). In contrast, Lat2 but not Mct8 is strongly expressed in thalamic nuclei (Thal). Elongated hybridization signals scattered throughout the CNS indicate a capillary localization of Oatp1c1 **(C1–C4)** and Lat1 **(D1–D4)**. In addition, Lat1 is present in the hippocampal CA3 neurons at postnatal day P6. Mct10 **(E1–E4)** is also temporarily expressed in hippocampal and cortical neurons. At P21, however, highest hybridization signal intensities are observed in white matter areas [e.g., in the cerebral peduncle (cp) or the hippocampal fimbria (fi)]. DG, dentate gyrus; DM, dorsomedial hypothalamic nucleus.

significantly reduced at P84 indicating an age-dependent decline in the respective transporter expression.

DISTRIBUTION PATTERN OF Mct10

Determination of Mct10 mRNA expression in the mouse brain at P6 and P12 revealed overall only very weak signal intensities in specific areas such as layer 6, and subplate neurons of the cerebral cortex as well as granule cells of the dentate gyrus and of the cerebellar cortex (**Figures 1E1–E4** and **2E1–E4**). At P21, strong signals could be detected throughout white matter regions suggesting that Mct10 is highly enriched in mature oligodendrocytes. In addition, Mct10 expression was clearly visible in the adult brain in neurons of the dentate gyrus and in distinct hypothalamic nuclei such as the dorsomedial nucleus (**Figures 2E1–E4**). Overall, Mct10 mRNA expression appeared to increase with increasing age of the animals.

DISCUSSION

Though the importance of TH transporters for proper TH action in the developing and mature CNS has been widely acknowledged, very limited information has been provided so far concerning the localization of TH transporter candidates both in the human as well as mouse CNS. In particular, the generation and analysis of Mct8 deficient mice have raised many questions since these animals lack any overt neurological symptoms, an unexpected finding in light of the pronounced expression of Mct8 in neuronal populations (15). Indeed, an area- as well as cell-type specific analysis of the thyroidal state revealed overall only a mild T3-deprivation such as in striatal cells (14), a normal T3-status in cerebellar neurons (14, 16) and very subtle changes in the cerebral cortex (29). The latter finding has been explained by the presence of Lat2 in cortical neurons that may compensate for the absence of Mct8 in the mouse brain (11). In support for this hypothesis, we could observe a pronounced neuronal expression of Lat2 throughout the cortex with highest signal intensities at P12. Mct8 and Lat2 may also co-operate in mediating TH transport in pyramidal cells of the hippocampus and in neurons of the hypothalamic paraventricular nucleus where transcripts for both transporters could be detected. Possibly, TRH expressing neurons are dependent on Mct8 and Lat2 for a proper negative feedback regulation within the hypothalamus–pituitary–thyroid axis. In this respect, it would be of most interest to study the TH-sensitivity of these neurons in Mct8/Lat2 double deficient animals.

Our analysis, however, also revealed striking differences in the Mct8 and Lat2 distribution pattern with the striatum, thalamus, and brain stem nuclei as the most evident examples. Based on these observations, we assume that besides Lat2, additional proteins must be present in the mouse brain that can facilitate the transmembrane passage of TH in neurons in the absence of Mct8.

One possible candidate is Mct10 that accepts in addition to aromatic amino acids TH as substrate as well (22). Mct10 protein

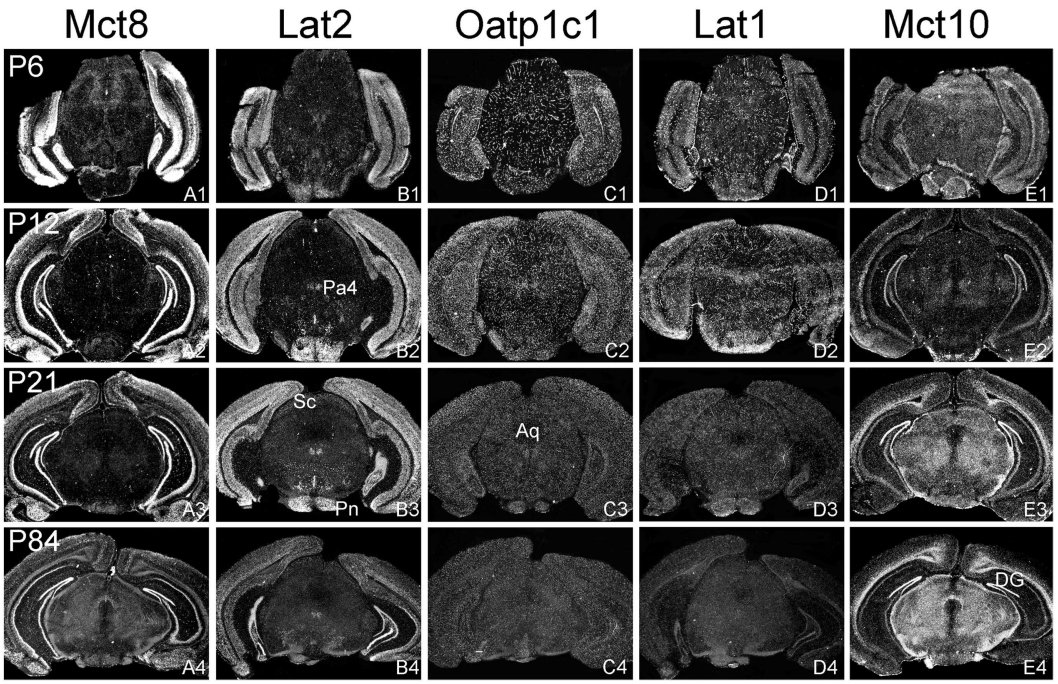

FIGURE 3 | Differential expression of TH transporters in midbrain regions. Whereas Mct8 mRNA expression is close to the detection limit in the midbrain area **(A1–A4)**, Lat2 specific hybridization signals **(B1–B4)** are observed in distinct nuclei such as the paratrochlear nucleus (Pa4) and the pontine nuclei (Pn). Weak signals for Lat2 are also found in the superior colliculi (Sc). Oatp1c1 **(C1–C4)** and Lat1 **(D1–D5)** exhibit a capillary expression pattern whereas Mct10 **(E1–E4)** is strongly expressed in the white matter and dentate gyrus neurons (DG). Aq, aqueduct.

was localized in neurons of the human hypothalamus (12), but its localization in the mouse brain has not been experimentally addressed yet. Here, we could detect Mct10 transcripts in neurons of the hippocampus where Mct10 showed an overlapping expression with Mct8. In contrast, Mct8 and Mct10 exhibited a complementary distribution pattern in the cerebral cortex, which makes a compensatory function of Mct10 in the absence of Mct8 rather unlikely. Moreover, our recent characterization of Mct10/Mct8 deficient animals did not reveal a pronounced TH deficiency in the CNS (30) indicating that at least in the developing murine brain, Mct10 does not significantly contribute to TH transport processes.

Intense Mct10 specific signals were found in white matter regions of the murine CNS at P21 and P84 suggesting that this transporter is highly expressed only in mature oligodendrocytes. It therefore remains to be investigated, which proteins are involved in supplying immature oligodendrocytes with TH particularly since TH represents a critical factor for proper differentiation of these cells (31, 32).

Previous studies of Mct8 ko mice demonstrated a critical role of this transporter in mediating the uptake of T3 via the BBB and/or the BCSFB (14, 16). This physiological function correlates with a strong expression of Mct8 in choroid plexus structures, tanycytes of the third ventricle as well as in capillary endothelial cells as evidenced by immunohistochemical studies (10, 11). Remarkably, Mct8 mRNA expression could only be detected by ISH in larger vessels while choroid plexus structures and tanycytes displayed strong Mct8 mRNA specific signals. The reason for the discrepancy between transcript and protein levels in capillary Mct8 expression cannot be solely related to possible limitations of the ISH procedure since smaller capillaries throughout the brains were successfully visualized with radioactive Oatp1c1 and Lat1 specific RNA probes. It therefore needs to be further assessed in a cell-specific manner how Mct8 expression is regulated on the transcriptional as well as on the translational level.

While in mice, inactivation of Oatp1c1 alone had only mild consequences on the thyroid state of the CNS (27), Mct8/Oatp1c1 dko mice displayed a highly diminished transport of T4 and T3 into the CNS, a finding that underscores the concerted function of Mct8 and Oatp1c1 in facilitating the brain entry of TH via the BBB and/or BCSF (20). Moreover, brain TH concentrations in Mct8/Oatp1c1 were reduced to 10% of the respective wild-type levels indicating a robust hypothyroid state in the CNS. However, it is currently unclear by which pathway the residual TH enters the brain in these animals. In this process, Lat1 may possibly be involved as our ISH analysis revealed a preferential expression of this transporter in capillary cells as well. The exact physiological contribution of Lat1 for TH traffic in the brain will only been unraveled following the generation and analysis of the respective mouse mutants that are either deficient in Lat1 alone or lack even all three TH transporters.

In summary, our ISH analysis revealed a distinct and unique temporal and spatial mRNA expression pattern for all five TH transporter candidates in the murine brain. Although we cannot

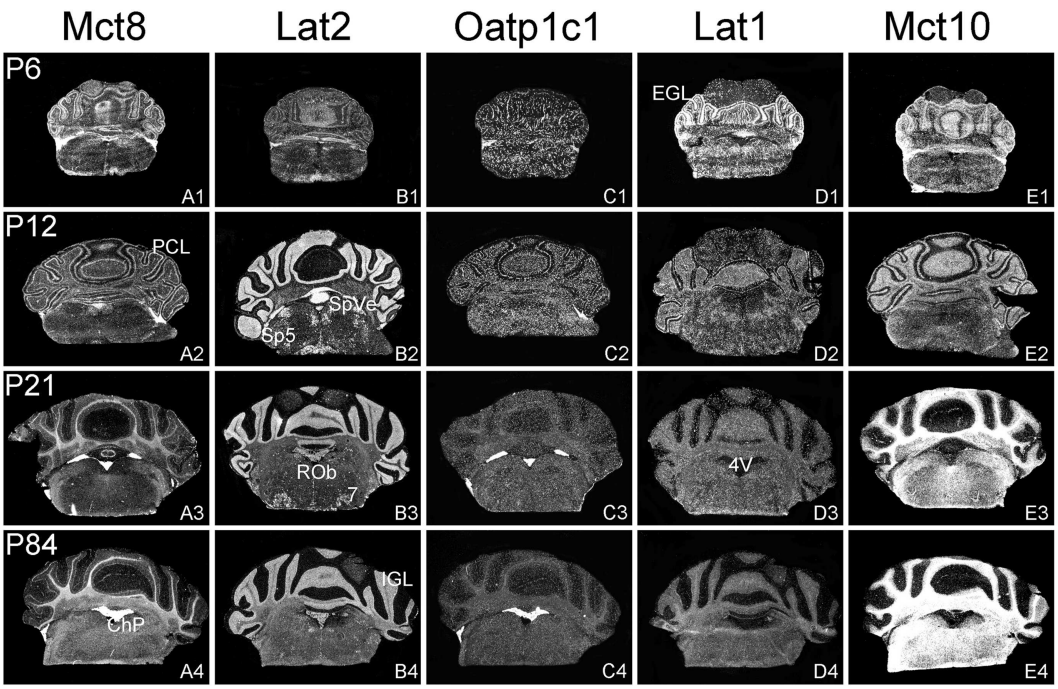

FIGURE 4 | Cerebellar and brainstem expression pattern of TH transporters. In the developing cerebellum, Mct8 specific hybridization signals can be found in the external granule cell layer (EGL) and the Purkinje cell layer (PCL) **(A1,A2)** whereas after weaning Mct8 mRNA expression is largely restricted to choroid plexus structures of the fourth ventricle (4V) **(A3,A4)**. In comparison, Lat2 mRNA levels are high in the internal granule cell layer (IGL) indicating that mature granule cells express this transporter **(B1–B4)**. Lat2 specific signals could also be found in various brainstem nuclei such as the facial nucleus (7), the raphe obscurus nucleus (ROb), the spinal 5 nucleus (Sp5), and the spinal vestibular nucleus (SpVe). Oatp1c1 is highly expressed in choroid plexus as well as in endothelial cells **(C1–C4)** where also Lat1 staining can be observed. In addition, Lat1 expression is found in the EGL and PCL of the cerebellar cortex at P6 **(D1–D4)**. Mct10 expression pattern indicates a pronounced localization in mature oligodendrocytes **(E1–E4)**.

provide any information about the respective protein levels and subcellular localization, our study will provide a solid ground for determining the cell-specific function of these transporters by taking advantage of conditional mouse mutants. Our data, however, also disclosed that additional TH transporter proteins need to be discovered in order to fully understand TH traffic in such a complex and important TH target organ as the CNS.

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REFERENCES

1. Hennemann G, Docter R, Friesema EC, de Jong M, Krenning EP, Visser TJ. Plasma membrane transport of thyroid hormones and its role in thyroid hormone metabolism and bioavailability. *Endocr Rev* (2001) **22**(4):451–76. doi:10.1210/er.22.4.451
2. Friesema EC, Jansen J, Milici C, Visser TJ. Thyroid hormone transporters. *Vitam Horm* (2005) **70**:137–67. doi:10.1016/S0083-6729(05)70005-4
3. Heuer H, Visser TJ. Minireview: Pathophysiological importance of thyroid hormone transporters. *Endocrinology* (2009) **150**(3):1078–83. doi:10.1210/en.2008-1518
4. Visser WE, Friesema EC, Jansen J, Visser TJ. Thyroid hormone transport in and out of cells. *Trends Endocrinol Metab* (2008) **19**(2):50–6. doi:10.1016/j.tem.2007.11.003
5. Friesema EC, Ganguly S, Abdalla A, Manning Fox JE, Halestrap AP, Visser TJ. Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. *J Biol Chem* (2003) **278**(41):40128–35. doi:10.1074/jbc.M300909200
6. 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**(1):168–75. doi:10.1086/380999
7. Friesema EC, Grueters A, Biebermann H, Krude H, von Moers A, Reeser M, et al. Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet* (2004) **364**(9443):1435–7. doi:10.1016/S0140-6736(04)17226-7
8. Schwartz CE, May MM, Carpenter NJ, Rogers RC, Martin J, Bialer MG, et al. Allan-Herndon-Dudley syndrome and the monocarboxylate transporter 8 (MCT8) gene. *Am J Hum Genet* (2005) **77**(1):41–53. doi:10.1086/431313
9. Friesema EC, Jansen J, Heuer H, Trajkovic M, Bauer K, Visser TJ. Mechanisms of disease: psychomotor retardation and high T3 levels caused by mutations in monocarboxylate transporter 8. *Nat Clin Pract Endocrinol Metab* (2006) **2**(9):512–23. doi:10.1038/ncpendmet0262
10. Roberts LM, Woodford K, Zhou M, Black DS, Haggerty JE, Tate EH, et al. Expression of the thyroid hormone transporters monocarboxylate transporter-8 (SLC16A2) and organic ion transporter-14 (SLCO1C1) at the blood-brain barrier. *Endocrinology* (2008) **149**(12):6251–61. doi:10.1210/en.2008-0378
11. Wirth EK, Roth S, Blechschmidt C, Holter SM, Becker L, Racz I, et al. 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**(30):9439–49. doi:10.1523/JNEUROSCI.6055-08.2009

12. Alkemade A, Friesema EC, Kalsbeek A, Swaab DF, Visser TJ, Fliers E. Expression of thyroid hormone transporters in the human hypothalamus. *J Clin Endocrinol Metab* (2011) **96**(6):E967–71. doi:10.1210/jc.2010-2750
13. 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**(9):4036–43. doi:10.1210/en.2006-0390
14. Trajkovic M, Visser TJ, Mittag J, Horn S, Lukas J, Darras VM, et al. Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8. *J Clin Invest* (2007) **117**(3):627–35. doi:10.1172/JCI28253
15. Heuer H, Maier MK, Iden S, Mittag J, Friesema EC, Visser TJ, et al. The monocarboxylate transporter 8 linked to human psychomotor retardation is highly expressed in thyroid hormone-sensitive neuron populations. *Endocrinology* (2005) **146**(4):1701–6. doi:10.1210/en.2004-1179
16. Ceballos A, Belinchon MM, Sanchez-Mendoza E, Grijota-Martinez C, Dumitrescu AM, Refetoff S, et al. Importance of monocarboxylate transporter 8 for the blood-brain barrier-dependent availability of 3,5,3'-triiodo-L-thyronine. *Endocrinology* (2009) **150**(5):2491–6. doi:10.1210/en.2008-1616
17. Pizzagalli F, Hagenbuch B, Stieger B, Klenk U, Folkers G, Meier PJ. Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter. *Mol Endocrinol* (2002) **16**(10):2283–96. doi:10.1210/me.2001-0309
18. Sugiyama D, Kusuhara H, Taniguchi H, Ishikawa S, Nozaki Y, Aburatani H, et al. Functional characterization of rat brain-specific organic anion transporter (Oatp14) at the blood-brain barrier: high affinity transporter for thyroxine. *J Biol Chem* (2003) **278**(44):43489–95. doi:10.1074/jbc.M306933200
19. Tohyama K, Kusuhara H, Sugiyama Y. Involvement of multispecific organic anion transporter, Oatp14 (Slc21a14), in the transport of thyroxine across the blood-brain barrier. *Endocrinology* (2004) **145**(9):4384–91. doi:10.1210/en.2004-0058
20. Mayerl S, Muller J, Bauer R, Richert S, Kassmann CM, Darras VM, et al. Transporters MCT8 and OATP1C1 maintain murine brain thyroid hormone homeostasis. *J Clin Invest* (2014) **124**(5):1987–99. doi:10.1172/JCI70324
21. Braun D, Kinne A, Brauer AU, Sapin R, Klein MO, Kohrle J, et al. Developmental and cell type-specific expression of thyroid hormone transporters in the mouse brain and in primary brain cells. *Glia* (2011) **59**(3):463–71. doi:10.1002/glia.21116
22. 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**(6):1357–69. doi:10.1210/me.2007-0112
23. Friesema EC, Docter R, Moerings EP, Verrey F, Krenning EP, Hennemann G, et al. Thyroid hormone transport by the heterodimeric human system L amino acid transporter. *Endocrinology* (2001) **142**(10):4339–48. doi:10.1210/endo.142.10.8418
24. Heuer H, Schafer MK, O'Donnell D, Walker P, Bauer K. Expression of thyrotropin-releasing hormone receptor 2 (TRH-R2) in the central nervous system of rats. *J Comp Neurol* (2000) **428**(2):319–36. doi:10.1002/1096-9861(20001211)428:2<319::AID-CNE10>3.3.CO;2-0
25. Visser WE, Friesema EC, Visser TJ. Minireview: thyroid hormone transporters: the knowns and the unknowns. *Mol Endocrinol* (2011) **25**(1):1–14. doi:10.1210/me.2010-0095
26. Franklin K, Paxinos G. *The mouse brain in stereotaxic coordinates*. San Diego: Academic Press (1997).
27. Mayerl S, Visser TJ, Darras VM, Horn S, Heuer H. Impact of Oatp1c1 deficiency on thyroid hormone metabolism and action in the mouse brain. *Endocrinology* (2012) **153**(3):1528–37. doi:10.1210/en.2011-1633
28. Schnell C, Shahmoradi A, Wichert SP, Mayerl S, Hagos Y, Heuer H, et al. The multispecific thyroid hormone transporter OATP1C1 mediates cell-specific sulforhodamine 101-labeling of hippocampal astrocytes. *Brain Struct Funct* (2013). doi:10.1007/s00429-013-0645-0
29. Morte B, Ceballos A, Diez D, Grijota-Martinez C, Dumitrescu AM, Di Cosmo C, et al. 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**(5):2381–7. doi:10.1210/en.2009-0944
30. Muller J, Mayerl S, Visser TJ, Darras VM, Boelen A, Frappart L, et al. Tissue-specific alterations in thyroid hormone homeostasis in combined mct10 and mct8 deficiency. *Endocrinology* (2014) **155**(1):315–25. doi:10.1210/en.2013-1800
31. Rodriguez-Pena A. Oligodendrocyte development and thyroid hormone. *J Neurobiol* (1999) **40**(4):497–512. doi:10.1002/(SICI)1097-4695(19990915)40:4<497::AID-NEU7>3.0.CO;2-#
32. Bernal J. Thyroid hormones and brain development. *Vitam Horm* (2005) **71**:95–122. doi:10.1016/S0083-6729(05)71004-9

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Thyroid hormone action: astrocyte–neuron communication

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Thyroid hormone (TH) action is exerted mainly through regulation of gene expression by binding of T3 to the nuclear receptors. T4 plays an important role as a source of intracellular T3 in the central nervous system via the action of the type 2 deiodinase (D2), expressed in the astrocytes. A model of T3 availability to neural cells has been proposed and validated. The model contemplates that brain T3 has a double origin: a fraction is available directly from the circulation, and another is produced locally from T4 in the astrocytes by D2. The fetal brain depends almost entirely on the T3 generated locally. The contribution of systemic T3 increases subsequently during development to account for approximately 50% of total brain T3 in the late postnatal and adult stages. In this article, we review the experimental data in support of this model, and how the factors affecting T3 availability in the brain, such as deiodinases and transporters, play a decisive role in modulating local TH action during development.

Keywords: thyroid hormone, type 2 deiodinase, astrocytes, fetal and postnatal brain, thyroid hormone transporters, T3 availability

THE MODEL

Thyroid hormone (TH) action is exerted mainly through regulation of gene expression by binding of T3 to the nuclear receptors (1, 2). T4 plays an important role as a source of intracellular T3 in the central nervous system via the action of the type 2 deiodinase (D2). This process is regulated physiologically. D2 activity in brain increases during development in correlation with the more T3 sensitive developmental period. D2 activity and T3 concentrations are synchronized in a spatial and temporal fashion to determine critical brain processes such as myelination, neuronal migration, glial differentiation, and neurogenesis. On the other hand, D2 activity is inversely regulated by T4, so that brain T3 levels fluctuates less than circulating TH levels, due to changes in D2 activity in response to changes in T4 availability at least at postnatal and adult stages (3).

Although neurons are the primary target of T3 actions, Guadaño-Ferraz et al. (4) demonstrated that D2 expression takes place predominantly, if not exclusively, in glial cells: the tanycytes (5) lining part of the third ventricle surface and in the astrocytes throughout the brain. The *Dio2* mRNA was not restricted to the cell body but was also present along the cellular processes. This observation indicated an important role for glial cells in TH homeostasis in the brain and a close coupling between glial cells and neurons in TH metabolism. According to these observations, the authors suggested a model of T3 availability to neural cells. On the one hand, circulating T4 and T3 would enter the brain through the blood–brain barrier (BBB). T4, upon entering the brain would reach the astrocytes through their end-feet in contact with the capillaries, and produce additional T3 by D2-mediated deiodination.

EVIDENCE FROM TRANSCRIPTOMIC DATA

Support for the glial specificity of Dio2 expression came from transcriptomic studies by Cahoy et al. (6). These authors performed transcriptomic studies in primary neural cells isolated from the mouse brain, without further manipulations to establish the patterns of gene expression representative from the different cell types *in vivo*. *Dio2* was enriched up to 50 times in the astrocytes, and therefore may be considered as a highly specific astrocyte gene. Despite this cellular specificity, there is evidence that *Dio2* is also expressed in cells other than the astrocytes in some situations. For example, in profound hypothyroidism in the rat, *Dio2* expression was also observed in a fraction of cerebral cortex interneurons (7). It was more recently demonstrated that astrocyte-specific inactivation of *Dio2* (GFAP–Cre–D2KO mice) reduced D2 activity in brain to less than 10% of the control (8).

EVIDENCE FROM PARACRINE INTERACTIONS *IN VITRO*

Freitas et al. (9) studied *in vitro* the evidence for a paracrine interaction between astrocytes and neurons. The goal was to check whether the T3 generated in glial cells was able to activate neuronal gene expression. This experimental system was based on an *in vitro* co-culture system of H4 human glioma cells expressing D2 and neuroblastoma cells. The two cell types were located in two adjacent compartments bathed with the same culture media. The authors demonstrated that upon incubation with T4, D2 activity in glial cells resulted in increased T3 production that reached neurons and promoted TH-responsive gene expression.

EVIDENCE FROM TRANSPORTER PATHOPHYSIOLOGY: DIRECT FUNCTIONAL DEMONSTRATION OF THE PARACRINE MODEL *IN VIVO*

A large body of evidence has accumulated in recent years demonstrating the crucial importance of transporters in mediating the cellular uptake of THs through the cell membranes (10). The most specific and physiologically relevant transporters for THs identified so far are the monocarboxylate transporter 8 (MCT8, SLC16A2), and the organic anion transporting polypeptide 1C1 (OATP1C1, SLCO1C1). MCT8 mutations cause an X-linked syndrome with severe psychomotor retardation and elevated serum T3 levels, indicating the importance of this transporter in TH availability to the brain (11, 12). Other transporters may also contribute to this process although their specific roles are less clear.

MCT8 transports T4 and T3 (13) and OATP1C1 exhibits a remarkable affinity and specificity toward T4 and rT3 (14, 15). Roberts et al. (16) reported the presence of Oatp1c1 protein in the abluminal side of endothelial capillary cells forming the BBB. The Oatp1c1 signal overlapped partially with aquaporin 4, a marker of astrocytes' end-feet, which are in contact with brain micro capillaries. Mct8/MCT8 is also expressed in the micro capillaries in rodent and human, but not in the astrocytes' end-feet. In addition, Mct8 is also expressed in neurons and choroid plexus (17).

Based on the preferences for TH transport and on the expression patterns of these two transporters, delivery of circulating T4 and T3 to the brain may be formulated in the following way: circulating T3 and T4, crossing the BBB through Mct8 would be delivered to the extracellular fluid, reaching directly the neural cells in the proximity of the micro capillaries. On the other hand, T4, but not T3, would be delivered directly to the astrocytes after Oatp1c1-mediated transport through the BBB. It is important to notice that OATP1C1 is poorly expressed in the BBB of human fetus (16) and adult primates (18) and therefore in the human brain, T4 transport is dependent on MCT8.

We provided experimental evidence in support for this model in rodents by measuring the relative effects of T4 and T3 on brain gene expression in *Mct8* knockout mice (Mct8KO) (19). T3 or T4 were administered to wild-type (WT) and to Mct8KO mice previously made hypothyroid, and expression of two neuronal target genes was measured in the cerebellum and striatum. Whereas T4 and T3 were similarly active in WT mice, the Mct8KO mice only responded to T4. The data suggested that the critical restriction to T3 transport in the absence of Mct8 is located at the BBB rather than at the plasma membrane of individual neurons, where other transporters can substitute for Mct8. The similarity in the effects of T4 in the Mct8-deficient and in the WT mice, suggested that T4 can reach the astrocytes in the Mct8-deficient mice through a different transporter, most probably Oatp1c1, and produce enough T3 to regulate neuronal gene expression through a paracrine interaction. Actually, the increased D2 activity present in the Mct8KO mice (20, 21), would facilitate this pathway in face of lower circulating T4, and restricted brain uptake of T3. The consequence is that the Mct8KO mice maintain brain gene expression similar to WT mice, with a few exceptions. Direct proof that D2 activity was indeed responsible for normal expression of most brain TH-regulated genes in the absence of Mct8 was also provided (22). In the Mct8KO mice, inactivation of D2 led to patterns of brain gene expression that were similar to that of severely hypothyroid mice, highlighting the importance of D2.

Therefore, the studies on the Mct8-deficient mice evidenced that the factors affecting T3 availability in the brain, namely deiodinases and transporters, play a decisive role in modulating local TH action. These studies also gave a functional direct demonstration of the critical role of astrocytes in regulating the amount of T3 available for neuronal uptake *in vivo*. The model also explains why the double inactivation of Mct8 and Oatp1c1 transporters (23) leads to a situation in the brain similar to hypothyroidism, as in the double Mct8 and Dio2 KO.

THE RELATIVE CONTRIBUTIONS OF DIRECT T3 UPTAKE FROM THE CIRCULATION AND THE LOCAL T3 PRODUCTION IN THE ASTROCYTES

POSTNATAL AND ADULT RODENTS

In the D2-deficient mice, brain T3 concentrations at postnatal day 15 are about half of normal, suggesting that at least from this postnatal age onward, 50% of the total brain T3 derives from direct T3 uptake from the circulation and another 50% derives from local T3 production in the astrocytes (24) (**Figure 1**). In support of this, Mct8 inactivation also leads to roughly 50% reduction of brain T3 (21).

Local production of T3 accounts for a much higher occupancy of brain nuclear receptors compared to the liver. Crantz et al. (25) estimated that local conversion of T4 to T3 accounted for 77% of the nuclear T3 receptor occupancy in the cerebral cortex and up to 37% in the cerebellum. Circulating T3 was responsible for about 20–25% of nuclear receptor occupancy. These authors concluded that the nuclear receptors were almost saturated by T3 in the cortex, and at 60% of total receptor capacity in the cerebellum. Full receptor saturation under euthyroid conditions may explain why TH-responsive genes in the cortex of euthyroid animals do not respond or do so modestly above the normal expression level after administration of excess T3. In studies of T3 administration to *Dio3* KO mice, we found that the response to high doses of T3 was higher in D3-deficient mice than in the WT euthyroid mice (26). Therefore, nuclear occupancy was not a limiting factor in gene responses to T3, and the reason why the euthyroid brain does not respond genomically to excess T3 is due to the protective action of D3.

Despite the importance of D2 for the local generation of T3, D2-deficient mice showed normal expression of several genes positively regulated by T3 (24). We confirmed these observations for additional genes, and showed that in the absence of D2, the expression levels of positively regulated genes are maintained by T3 from the circulation (22). However, expression of the negatively regulated genes is more frequently affected by D2 inactivation, suggesting that they are sensitive to the source of T3. The mechanism underlying these observations is unknown, and somehow the source of T3 may influence directly or indirectly the cellular T3 content.

THYROID HORMONE TRANSPORT IN THE FETAL BRAIN

The fetal brain depends almost entirely from T4 monodeiodination, so that most brain T3 is produced locally from T4 (**Figure 1**). In the human fetal brain, despite the presence of T3 receptors

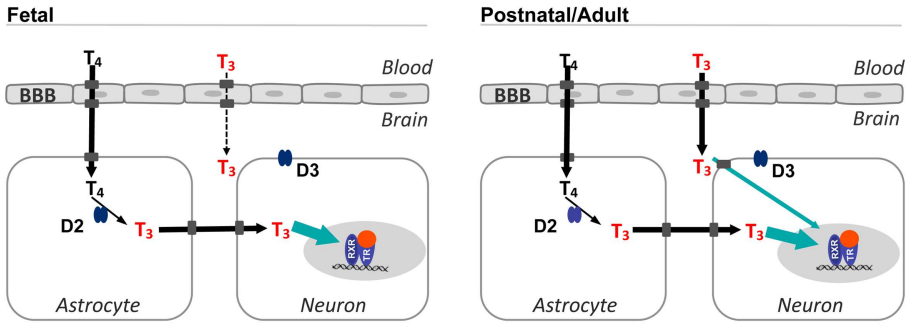

FIGURE 1 | Sources of T3 in the fetal and postnatal brain. T4 from the circulation crosses the BBB and reaches the astrocytes and is converted to T3 by type 2 deiodinase. This is the main source of T3 in the fetal brain. In postnatal and adult animals, T3 from the circulation can also access the brain. The proportion of T3 from the circulation increases up to 50% in the late postnatal stages. *In situ* T3 production accounts for a high occupancy of thyroid hormone receptors (green arrow), and is also important for expression of genes regulated negatively by thyroid hormone. Transporters in the cell membranes are represented by gray squares, without naming any specific transporter. Although in the rodent brain, the BBB expresses Mct8 and Oatp1c1, the latter is not present in the primate BBB. T4 enters the astrocytes most likely through Oatp1c1. Passage of T3 from astrocytes to neurons is facilitated by Mct8 and also other transporters, since in Mct8 KO mice there is no apparent restriction for the passage of astrocytic T3 to neurons. Type 3 deiodinase is localized in the plasma membrane of neurons. BBB, blood–brain barrier; D2, type 2 deiodinase; D3, type 3 deiodinase; TR, thyroid hormone receptor; RXR, 9-cis retinoic acid receptor.

and T4 in tissues by the second trimester, and even before, only the brain showed a significant T3 concentration in the nucleus as compared with other tissues (27). This observation indicates that the main source of T3 in the fetal brain is local T4 deiodination. In support of this idea, D2 activity rises in the human cerebral cortex during the second trimester in parallel with T3 concentrations (28).

In the rat (29, 30), physiological doses of T4 administered to hypothyroid pregnant rats could normalize T3 concentrations in the brain and increase neuronal gene expression. In contrast, administration of even large doses of T3 to the mother failed to increase T3 concentration in the fetal brain despite reaching other tissues, and was not able to normalize fetal gene expression. The rat fetal brain contains significant D2 activity (31) making it plausible that most if not all brain T3 is produced locally.

WHY DOES CIRCULATING T3 CANNOT ENTER THE FETAL BRAIN?

The reason why the fetal brain is apparently impermeable to circulating T3 is unknown. A possible explanation could be a lack of expression of the Mct8 transporter. To address this issue, the expression of Mct8 and of the specific T4 transporter Oatp1c1 were analyzed by confocal microscopy in the prenatal rat cortex. Both proteins were present in the brain capillaries and in the epithelial cells of the choroid plexus (30).

If T3 cannot enter the brain despite expression of Mct8, what is the role of this transporter in the fetal brain? It is possible that Mct8 at early stages of development has a more prominent role in TH efflux from the brain (32, 33) as proposed in the liver for Mct8 and Mct10 (34). Another possibility is that circulating T3 crossing the BBB through Mct8-mediated transport is deposited directly in the extracellular fluid where it could be rapidly degraded by neuronal D3 activity (35). D3 is abundantly expressed in fetal tissues including the brain (36, 37), where it is expressed in neurons (38, 39) and serves as a fine regulator of tissue TH concentrations. For example, in the human fetal brain, D3 activity in the cerebellum prevents the accumulation of T3 during mid-gestation (28), at the same time that D2 activity is responsible for T3 accumulation in the cortex. Hernandez et al. (40) demonstrated that D3 plays a critical role in maintaining low levels of TH during fetal and early neonatal life in mice. During postnatal development, disruption of the *Dio3* gene increases basal expression of the T3-target gene *Hr* (41). The catalytic site of D3 was earlier proposed to face the extracellular fluid (42, 43). If this was true, T3 in the extracellular fluid of the fetal brain would be easily accessible to D3-mediated degradation. However, more recent evidence derived from *Dio3* transfection experiments indicates that D3 substrates need to be internalized for degradation (44). Even so, it may be speculated that the topography of D3 localization in the plasma membrane could allow easier and faster degradation of substrates crossing the neuronal membrane from the extracellular fluid. Within this context, one attractive, but still speculative hypothesis is that the increased proportion of T3 entering the brain from the circulation, taking place from fetal to postnatal stages, is due a decrease of D3 activity. The contribution of systemic T3 would then increase in parallel to account for approximately 50% of the total brain T3.

LOCAL GENERATION OF T3 FROM T4 IN THE CONTEXT OF ASTROGLIAL MATURATION

The contention that in the fetal brain most T3 derives from T4 has to be examined in the context of D2 activity and maturation of D2-expressing astrocytes during development. The main surge of D2 activity in the rat brain is postnatal, with a peak around postnatal day 15, a time in which the highest T3 concentration in the brain is reached. In the fetal brain, D2 activity is low, and shows a discrete peak just before birth, at prenatal days 18–21 (31, 45). Consequently, brain T3 concentrations during the perinatal period are low, about half to one-third of adult rats.

Some aspects of the model are not well-understood, and should be refined to take into account several factors. One is astrocyte

development, which is mainly postnatal (46), increasing in number during postnatal stages, and following a pattern similar to D2 activity. Although the role of astrocytes during the fetal and early postnatal periods has been questioned (47), native astrocytes isolated from the P1 mouse brain already contain a high concentration of *Dio2* mRNA (6). It may be that the small population of astrocytes present in the last few days of fetal stages has an important role in the local formation of T3 in the brain. Another difficulty is to extrapolate the model to the human situation. The human brain expresses little OATP1C1 in the BBB, and T4 and T3 transport apparently rely exclusively on MCT8. Therefore, entry of T4 to the astrocytes has to take place through a different transporter. Specifically addressing the patterns of thyroid hormone transporter, and deiodinases in the fetal and postnatal human brain should shed light on these issues.

REFERENCES

1. Bernal J, Guadano-Ferraz A, Morte B. Perspectives in the study of thyroid hormone action on brain development and function. *Thyroid* (2003) **13**:1005–12. doi:10.1089/105072503770867174
2. Bernal J. Thyroid hormone receptors in brain development and function. *Nat Clin Pract Endocrinol Metab* (2007) **3**:249–59. doi:10.1038/ncpendmet0424
3. Croteau W, Davey JC, Galton VA, St Germain DL. Cloning of the mammalian type II iodothyronine deiodinase. A selenoprotein differentially expressed and regulated in human and rat brain and other tissues. *J Clin Invest* (1996) **98**:405–17. doi:10.1172/JCI118806
4. Guadaño-Ferraz A, Obregon MJ, St Germain DL, Bernal J. The type 2 iodothyronine deiodinase is expressed primarily in glial cells in the neonatal rat brain. *Proc Natl Acad Sci U S A* (1997) **94**:10391–6. doi:10.1073/pnas.94.19.10391
5. Tu HM, Kim SW, Salvatore D, Bartha T, Legradi G, Larsen PR, et al. Regional distribution of type 2 thyroxine deiodinase messenger ribonucleic acid in rat hypothalamus and pituitary and its regulation by thyroid hormone. *Endocrinology* (1997) **138**:3359–68. doi:10.1210/endo.138.8.5318
6. Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, et al. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J Neurosci* (2008) **28**:264–78. doi:10.1523/JNEUROSCI.4178-07.2008
7. Guadaño-Ferraz A, Escamez 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**:3430–9.
8. Fonseca TL, Werneck-De-Castro JP, Castillo M, Bocco BM, Fernandes GW, Mcaninch EA, et al. Tissue-specific inactivation of type 2 deiodinase reveals multilevel control of fatty acid oxidation by thyroid hormone in the mouse. *Diabetes* (2014) **63**:1594–604. doi:10.2337/db13-1768
9. Freitas BC, Gereben B, Castillo M, Kallo I, Zeold A, Egri P, et al. Paracrine signaling by glial cell-derived triiodothyronine activates neuronal gene expression in the rodent brain and human cells. *J Clin Invest* (2010) **120**:2206–17. doi:10.1172/JCI41977
10. Friesema EC, Jansen J, Milici C, Visser TJ. Thyroid hormone transporters. *Vitam Horm* (2005) **70**:137–67. doi:10.1016/S0083-6729(05)70005-4
11. 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**:168–75. doi:10.1086/380999
12. Friesema EC, Grueters A, Biebermann H, Krude H, von Moers A, Reeser M, et al. Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet* (2004) **364**:1435–7. doi:10.1016/S0140-6736(04)17226-7
13. Friesema EC, Ganguly S, Abdalla A, Manning Fox JE, Halestrap AP, Visser TJ. Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. *J Biol Chem* (2003) **278**:40128–35. doi:10.1074/jbc.M300909200
14. Pizzagalli F, Hagenbuch B, Stieger B, Klenk U, Folkers G, Meier PJ. Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter. *Mol Endocrinol* (2002) **16**:2283–96. doi:10.1210/me.2001-0309
15. Sugiyama D, Kusuhara H, Taniguchi H, Ishikawa S, Nozaki Y, Aburatani H, et al. Functional characterization of rat brain-specific organic anion transporter (Oatp14) at the blood-brain barrier: high affinity transporter for thyroxine. *J Biol Chem* (2003) **278**:43489–95. doi:10.1074/jbc.M306933200
16. Roberts LM, Woodford K, Zhou M, Black DS, Haggerty JE, Tate EH, et al. Expression of the thyroid hormone transporters monocarboxylate transporter-8 (SLC16A2) and organic ion transporter-14 (SLCO1C1) at the blood-brain barrier. *Endocrinology* (2008) **149**:6251–61. doi:10.1210/en.2008-0378
17. Heuer H, Maier MK, Iden S, Mittag J, Friesema EC, Visser TJ, et al. The monocarboxylate transporter 8 linked to human psychomotor retardation is highly expressed in thyroid hormone-sensitive neuron populations. *Endocrinology* (2005) **146**:1701–6. doi:10.1210/en.2004-1179
18. Ito K, Uchida Y, Ohtsuki S, Aizawa S, Kawakami H, Katsukura Y, et al. Quantitative membrane protein expression at the blood-brain barrier of adult and younger cynomolgus monkeys. *J Pharm Sci* (2011) **100**:3939–50. doi:10.1002/jps.22487
19. Ceballos A, Belinchon MM, Sanchez-Mendoza E, Grijota-Martinez C, Dumitrescu AM, Refetoff S, et al. Importance of monocarboxylate transporter 8 for the blood-brain barrier-dependent availability of 3,5,3'-triiodo-L-thyronine. *Endocrinology* (2009) **150**:2491–6. doi:10.1210/en.2008-1616
20. 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**:4036–43. doi:10.1210/en.2006-0390
21. Trajkovic M, Visser TJ, Mittag J, Horn S, Lukas J, Darras VM, et al. Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8. *J Clin Invest* (2007) **117**:627–35. doi:10.1172/JCI28253
22. Morte B, Ceballos A, Diez D, Grijota-Martinez C, Dumitrescu AM, Di Cosmo C, et al. 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**:2381–7. doi:10.1210/en.2009-0944
23. Mayerl S, Muller J, Bauer R, Richert S, Kassmann CM, Darras VM, et al. Transporters MCT8 and OATP1C1 maintain murine brain thyroid hormone homeostasis. *J Clin Invest* (2014) **124**:1987–99. doi:10.1172/JCI70324
24. Galton VA, Wood ET, St Germain EA, Withrow CA, Aldrich G, St Germain GM, et al. Thyroid hormone homeostasis and action in the type 2 deiodinase-deficient rodent brain during development. *Endocrinology* (2007) **148**:3080–8. doi:10.1210/en.2006-1727
25. 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**:367–75. doi:10.1210/endo-110-2-367
26. 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**:2919–28. doi:10.1210/en.2011-1905
27. Bernal J, Pekonen F. Ontogenesis of the nuclear 3,5,3'-triiodothyronine receptor in the human fetal brain. *Endocrinology* (1984) **114**:677–9. doi:10.1210/endo-114-2-677
28. Kester MH, Martinez de Mena R, Obregon MJ, Marinkovic D, Howatson A, Visser TJ, et al. Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. *J Clin Endocrinol Metab* (2004) **89**:3117–28. doi:10.1210/jc.2003-031832
29. Calvo R, Obregon MJ, Ruiz de Ona 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**:889–99. doi:10.1172/JCI114790
30. 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**(4):1713–21. doi:10.1210/en.2010-1014
31. Ruiz de Ona C, Obregon 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**:588–94. doi:10.1203/00006450-198811000-00010
32. Ferrara AM, Liao XH, Gil-Ibanez P, Marcinkowski T, Bernal J, Weiss RE, et al. Changes in thyroid status during perinatal development of MCT8-deficient male mice. *Endocrinology* (2013) **154**:2533–41. doi:10.1210/en.2012-2031
33. Núñez B, Martínez de Mena R, Obregon MJ, Font-Llitjós M, Nunes V, Palacín M, et al. Cerebral cortex hyperthyroidism of newborn Mct8-deficient mice

transiently suppressed by Lat2 inactivation. *Plos One* (2014) **9**(5):e96915. doi:10.1371/journal.pone.0096915
34. Muller J, Mayerl S, Visser TJ, Darras VM, Boelen A, Frappart L, et al. Tissue-specific alterations in thyroid hormone homeostasis in combined Mct10 and Mct8 deficiency. *Endocrinology* (2014) **155**:315–25. doi:10.1210/en.2013-1800
35. Hernandez A. Structure and function of the type 3 deiodinase gene. *Thyroid* (2005) **15**:865–74. doi:10.1089/thy.2005.15.865
36. 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**:1208–14. doi:10.1172/JCI110136
37. Galton VA, Martinez E, Hernandez 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**:979–87. doi:10.1172/JCI6073
38. 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**:784–90. doi:10.1210/endo.140.2.6486
39. Escamez MJ, Guadano-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**:5443–6. doi:10.1210/endo.140.11.7244
40. 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**:476–84. doi:10.1172/JCI26240
41. 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**:5550–8. doi:10.1210/en.2010-0450
42. Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, et al. Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocr Rev* (2008) **29**:898–938. doi:10.1210/er.2008-0019
43. Baqui M, Botero D, Gereben B, Curcio C, Harney JW, Salvatore D, et al. Human type 3 iodothyronine selenodeiodinase is located in the plasma membrane and undergoes rapid internalization to endosomes. *J Biol Chem* (2003) **278**:1206–11. doi:10.1074/jbc.M210266200
44. Jansen J, Friesema EC, Kester MH, Schwartz CE, Visser TJ. Genotype-phenotype relationship in patients with mutations in thyroid hormone transporter MCT8. *Endocrinology* (2008) **149**:2184–90. doi:10.1210/en.2007-1475
45. Obregon MJ, Ruiz de Ona 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**:2663–73. doi:10.1210/endo-129-5-2663
46. Bushong EA, Martone ME, Ellisman MH. Maturation of astrocyte morphology and the establishment of astrocyte domains during postnatal hippocampal development. *Int J Dev Neurosci* (2004) **22**:73–86. doi:10.1016/j.ijdevneu.2003.12.008
47. Daneman R, Zhou L, Kebede AA, Barres BA. Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature* (2010) **468**:562–6. doi:10.1038/nature09513

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Thyroid hormone role on cerebellar development and maintenance: a perspective based on transgenic mouse models

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Cerebellum development is sensitive to thyroid hormone (TH) levels, as THs regulate neuronal migration, differentiation, and myelination. Most effects of THs are mediated by the thyroid hormone receptor (TR) isoforms TRβ1, TRβ2, and TRα1. Studies aimed at identifying TH target genes during cerebellum development have only achieved partial success, as some of these genes do not possess classical TH-responsive elements, and those that do are likely to be temporally and spatially regulated by THs. THs may also affect neurodevelopment by regulating transcription factors that control particular groups of genes. Furthermore, TH action can also be affected by TH transport, which is mediated mainly by monocarboxylate transporter family members. Studies involving transgenic animal models and genome-wide expression analyses have helped to address the unanswered questions regarding the role of TH in cerebellar development. Recently, a growing body of evidence has begun to clarify the molecular, cellular, and functional aspects of THs in the developing cerebellum. This review describes the current findings concerning the effects of THs on cerebellar development and maintenance as well as advances in the genetic animal models used in this field.

Keywords: thyroid hormones, genes, cerebellum, brain development, animal models

INTRODUCTION

The thyroid hormones (THs) thyroxine (T_4) and 3,5,3′-triiodothyronine (T_3) are essential for embryonic development and play critical roles in cellular metabolism, acting primarily through the stimulation of oxygen consumption and basal metabolic rate (1, 2). THs are necessary for proper central nervous system (CNS) development, and they have long been known to regulate neuronal differentiation and migration, synaptogenesis, and myelination (3–6). The cerebellum is located near the rear of the brain stem at the midbrain–hindbrain junction, and this structure is generally thought to coordinate proprioceptive–motor functions, although more recently, it has also been associated with neurocognition (7, 8). The cerebellum was one of the first targets of THs to be identified, and it is a useful model for studying the mechanisms by which THs influence the CNS. In particular, the cerebellum has a relatively homogenous and simple structure with a well-characterized laminar organization and a small number of cell types that develop within spatially defined regions (9–11).

The majority of TH actions are mediated through the binding of T_3 to nuclear thyroid hormone receptors (TRs), which act as ligand-modulated transcription factors that modify the expression of target genes (12). Fundamentally, TH nuclear signaling is mediated by interactions between TRs and specific DNA sequences known as thyroid response elements (TREs), which associate with a variety of co-factors within the regulatory regions of target genes (12, 13). TR isoforms are expressed in several brain regions, including the cerebellum (14, 15). However, the target genes of THs and the cells that express genes likely to be involved in cerebellar development and maintenance are still not well-established (6, 16).

In addition to the classical roles of TH in the nucleus, TH can also initiate rapid effects at the cell surface, within mitochondria and via cytoplasmic TRs (17, 18). The fact that brain development in TR knockout (KO) animals is only slightly affected (19) suggests the existence of non-genomic morphogenic roles for TH in the CNS. One of the best characterized non-genomic roles for TH in the brain is illustrated by the induction of actin polymerization in astrocytes by T_4 *in vitro* (20), which is very important for the organization of extracellular neural guidance molecules during neurodevelopmental processes. Finally, TH metabolism and transport, which are mediated mainly by deiodinases (21) and monocarboxylate transporters (22, 23), respectively, have also been shown to be important for cerebellar function.

The aims of this review are to briefly describe the current knowledge concerning the effects of THs on cerebellar development and functional maintenance as well to summarize advances in the genetic animal models used in this field.

THE INFLUENCE OF THs ON CEREBELLAR ONTOGENESIS

In humans, T_3, T_4, and TRs are already present within the developing cortex prior to the onset of fetal thyroid gland activity, or gestational week 12, which suggests an important role for maternal TH during this critical window of brain development (24–27). Congenital hypothyroidism leads to structural and intellectual impairment in infants (28). Furthermore, TH administration to human infants with congenital hypothyroidism immediately after birth was shown to promote near-normal intellectual development (29). The majority of studies on the role of THs in neurodevelopment have been carried out in rodent models in which THs,

deiodinases, and TRs are present prior to the onset of fetal TH synthesis and secretion (30, 31). *Paired box 8* (*Pax8*) KO mice are a commonly used animal model for studying the effects of postnatal TH on CNS development, as Pax8 is an essential transcription factor for thyroid follicular cell differentiation, and its absence leads to thyroid gland dysgenesis (32). Therefore, the *Pax8*-KO mouse is a model for congenital hypothyroidism that displays extensive abnormalities in cerebellar development, resulting in an ataxic phenotype (32–34) (**Table 1**).

Rodent cerebellar development is complete within the first 2–3 weeks after birth, when the cerebellar foliation process, which encompasses the transition from a smooth cerebellar surface to an X lobule cerebellum, is completed (7). It has long been known that cerebellar ontogenesis is closely linked to TH regulation (60–62), although the molecular mechanisms through which THs modulate this process remain unclear. Hypothyroidism results in a number of morphological alterations in the cerebellum, including increased neuronal death within the internal granular layer (IGL), increased perdurance of the external granular layer (EGL), defects in granular cell migration, impaired Purkinje cell dendritogenesis, delayed myelination, defects in the late differentiation pattern of Golgi interneurons and mossy fibers, reduced protrusions of Bergmann glial cells, and increased cell apoptosis (9, 46, 63–65). TH administration prior to the end of postnatal week 2 prevented these structural changes. Moreover, the expression levels of neurotrophins and growth factors, such as BDNF, NT3, and EGF, as well as cell adhesion molecules, such as NCAM and L1, are modified by TH in the developing cerebellum (63, 66–68). For example, TH was shown to promote cerebellar neuronal migration and the differentiation of Bergmann glia by inducing EGF secretion (69).

PERSPECTIVES FROM TRANSGENIC MOUSE MODELS

T_3 and T_4 enter the cell through plasma membrane transporters, including the monocarboxylate transporter family members MCT8 and MCT10, organic anion transporting peptides (OATP), and carriers of L-amino acids (LATS) (70, 71). Recent studies have indicated that TH transporters such as MCT8, which are found in a subset of neuronal populations (23), may play critical roles in neurodevelopment processes mediated by THs. Patients harboring inactivating mutations in the MCT8 gene (*Slc16a2*) exhibit Allan–Herndon–Dudley syndrome, which is characterized by psychomotor retardation, lack of speech development, increased serum T_3 concentrations, and low T_4 levels (72, 73).

Although MCT8-KO mice have been generated, they do not display the same neurological abnormalities observed in human patients (**Table 1**). This phenomenon is likely due to the presence of other neuronal TH transporters, such as OATP14, LAT1, and LAT2, during earlier stages of mouse brain development that compensate for the absence of MCT8 (36, 74). However, another possible explanation for the difference between the mouse and human phenotypes is that human MCT8 is necessary for the transport of an unknown signaling molecule necessary for CNS development, which is consistent with clinical evidence indicating that the neurological syndromes observed in patients with MCT8 mutations are more severe than those observed in patients with congenital hypothyroidism (36). A recent study performed in MCT8-KO mice demonstrated that 3,5,3′,5′-tetraiodothyroacetic acid (tetrac), a T_4 metabolite that is not transported by MCT8 or OATP1C1, is capable of replacing TH during brain development (35). Tetrac can be converted into 3,3′,5-triiodothyroacetic acid (triac) by deiodinase type 2, which can subsequently interact with TRs, thereby replacing T_3 activity. Indeed, treatment of MCT8-KO mice with tetrac led to improvements in TH-dependent neuronal differentiation in the striatum, cortex, and cerebellum during the first three postnatal weeks.

A mouse model lacking LAT2 (*Slc7a8*) was generated to further characterize the role of this transporter in TH physiology. However, LAT2-KO mice exhibited normal cerebral and cerebellar development, with the exception of slight defects in movement coordination on rotarod tests (40) (**Table 1**).

The iodothyronine deiodinase enzymes D1 (*Dio1*) and D2 (*Dio2*) modulate the intracellular availability of the active hormone T_3. In particular, D2 catalyzes the conversion of T_4 to T_3, whereas D3 inactivates T_4 and T_3 by converting them to T_2 and reverse T_3 (rT_3), respectively (75). Studies have demonstrated that nearly 80% of T_3 is generated by local conversion within the brain (3, 5) through the activity of D2, which is primarily found in astrocytes (41). Therefore, the presence of D2 together with increased levels of T_3 suggests a role for D2 in supplying the developing brain with T_3 derived from maternal T_4. However, some unexpected findings in *Dio2*-KO mice are inconsistent with the hypothesis that D2 is essential for all TH-dependent neurodevelopment processes.

Although *Dio2*-KO mice display elevated brain T_4 levels and reduced T_3 content, surprisingly, the observed neurological impairments, which included changes in the cerebellar expression of TH-dependent genes and behavioral defects, were found to be mild compared with those observed in hypothyroidism (42, 76). These data suggest that decreased local T_3 production can be largely compensated for by increased T_3 uptake from circulation, and indeed, this was later confirmed by experiments carried out in double *Dio1*/*Dio2*-KO mice, which demonstrated normal serum T_3 concentrations and only mild neurological phenotypes (21). On the other hand, *Dio3*-KO animals were characterized by high T_3 levels during perinatal development, which induced the upregulation of TH-responsive genes in the cerebellum (43, 44). Recently, it was reported that *Dio3*-KO mice exhibited impaired cerebellar foliation, early premature disappearance of the EGL, rapid expansion of the molecular layer, and abnormal locomotor behavior. Furthermore, the cerebellar phenotypes of these mice could be partially rescued by deletion of the TRα1 isoform (45) (**Table 1**).

The majority of TH functions are mediated through nuclear TRs, which are members of a superfamily of ligand-modulated transcription factors that can either upregulate or downregulate target gene transcription (2). The consensus for positively regulated genes is that TRs bind to activating TREs both in the presence and absence of T_3. In the absence of T_3, TR represses target gene transcription by recruiting co-repressors, whereas in the presence of T_3, co-repressors are released and co-activators are recruited, leading to transcriptional up regulation (1, 12). In mammals, two different genes encode at least three high-affinity TRs: TR-β1 (*Thrb*), TR-β2 (*Thrb*), and TR-α1 (*Thra*) (77). TR-α1 is the isoform that is predominantly expressed both prenatally and postnatally throughout the brain, including the developing cerebellum, and it is responsible for nearly 80% of total receptor T_3 binding (14, 78, 79). In contrast, TR-β expression is confined to a

Table 1 | Summary of mutant animal models and their cerebellar phenotypes.

Animal model	Etiology	HPT axis	Brain TH state	Cerebellar phenotype	Locomotor behavior	Reference
Pax8-KO	Pax8 knockout	Thyroid gland dysgenesis	Increased TRH and TSH expression; elevated cerebellar D2 activity; decreased cerebellar D3 activity	Increased cell number in the EGL; reduced dendritic growth in Purkinje cells	Ataxic phenotype	(32, 33, 35)
Slc16a2 KO	MCT8 knockout	Elevated serum levels of T_3 and TSH; decreased serum levels of T_4	Reduced T_3 and T_4 brain content; increased TRH expression; increased cerebellar D2 activity; decreased cerebellar D3 activity	Milder neurological phenotype than that observed in patients; no alterations in Purkinje cells	Locomotor activity similar to WT mice	(35–38)
Pax8/Slc16a2 double KO	Pax8 and MCT8 knockout	Thyroid gland dysgenesis	Increased TRH and TSH expression; increased cerebellar D2 activity; decreased cerebellar D3 activity	Reduced dendritic arborization; thinner molecular layer		(35)
Slco1c1 KO	OATP1C1 knockout	Normal serum T_3 and T_4 levels	Mild decrease in T_4 brain content; normal T_3 brain content	Normal Purkinje cell morphology	Normal motor activity on rotarod test	(39)
Slco1c1/Slc16a2 double KO	OATP1C1 and MCT8 knockout	Elevated serum levels of T_3 and TSH; decreased serum levels of T_4	Brain-specific hypothyroidism increased TRH expression; elevated cerebellar D2 activity; reduced cerebellar D3 activity	Impaired arborization and dendritic growth of Purkinje cells at P12; no alterations in Purkinje cells at P33 or P120	Impaired motor coordination and locomotor activity	(38)
Slc7a8 KO	LAT2 knockout	Normal serum T_3, T_4, and TSH levels	Normal TSH expression; normal pituitary D2 expression; normal cerebellar D3 expression	Normal cerebellar gene expression and morphology	Mildly impaired movement coordination on rotarod test	(40)
Dio2-KO	D2 knockout	Normal serum T_3 levels; elevated serum T_4, and TSH levels	Decreased T_3 brain content; increased brain D3 activity	Milder alterations in cerebellar TH-responsive genes (*Srg1* and *Hr*) than in hypothyroidism		(41, 42)
Dio3-KO	D3 knockout	Increased serum T_3 levels during perinatal development	Brain thyrotoxicosis; increased cerebellar D2 activity; reduced cerebellar D3 activity	Upregulated cerebellar TH-responsive genes (*Hr*); impaired cerebellar foliation; early dissipation of EGL; rapid expansion of the molecular layer	Defective locomotor activity on vertical pole and rotarod test	(43–45)
Thra$^{-/-}$	TRα1 deletion	Normal serum T_3 levels; slightly decreased serum T_4 levels; reduced serum TSH levels	Decreased TSHα expression; increased TSHβ expression	Non-hypothyroid cerebellar phenotype	Normal locomotor activity	(46, 47)
Thrb$^{-/-}$	All TRβ deletion	Increased levels of TSH, T_3, and T_4	Increased T_3 brain content decreased TSH expression	No alterations in TH-responsive genes in the cerebellum	No behavioral defects	(48, 49)

(Continued)

Table 1 | Continued

Animal model	Etiology	HPT axis	Brain TH state	Cerebellar phenotype	Locomotor behavior	Reference
Thrb Δ337T	TRβ mutation	Elevated levels of T_3, T_4, and TSH	Hypothyroid-like brain (low levels of TH-responsive genes BDNF and *Pcp2*)	Impaired cerebellar foliation; altered laminar organization; abnormal Purkinje cell dendritogenesis; reduced Bergmann glia fibers; reduced cerebellar gene expression (*Pcp2*)	Severe impairment in balance and coordination	(50, 51)
Thra PV	TRα1 mutation	Mild increase of T_3, T_4, and TSH levels		Reduced cerebellar gene expression (*Srg1*)		(52)
Thra R384C	TRα1 mutation	Normal serum levels of T_4, T_3	Normal TSH expression	Delayed migration of EGL to IGL; mild alterations of Purkinje cells	Reduced locomotor activity	(53, 54)
Thra L400R	TRα1 mutation	Normal serum levels of T_4, T_3	Normal TSH expression Hypothyroid-like brain (low levels of TH-responsive genes)	Late granule cell differentiation pattern similar to congenital hypothyroidism; mild alterations of Purkinje cell arborization; low expression of TH-responsive genes (*Hr* and *Pcp2*); delayed loss of Purkinje cells axonal regenerative capacity; impaired differentiation of Purkinje cells and Bergmann glia		(55–58)
Ncoa1$^{-/-}$	SRC-1 deletion	Elevated TSH, T_4, and T_3 levels		Delayed Purkinje cells development and maturation	Reduced motor coordination and strength	(59)

BDNF, brain-derived neurotrophic factor; EGL, external granular layer; IGL, internal granular layer; Srg1, synaptotagmin-related gene 1, Hr, hairless; Pcp2, Purkinje cell protein 2.

few postnatal neuronal populations, including the paraventricular hypothalamus, cerebellar Purkinje cells, and hippocampal pyramidal and granule cells (80, 81). In rodents, TR-α1 is already present at E11.5 in the neural tube and at E12.5 in the diencephalon and ventral rhombencephalon (14). Both TRα and TRβ are expressed in the cerebellum. TRα is primarily expressed in the early cerebellar neurepithelium, granular cell precursors, and later in the transient EGL, whereas TRβ is predominantly expressed during later stages, notably in the Purkinje cell layer (PCL) and in deep internal layers (14, 81, 82) (**Figure 1**).

Thra- and *Thrb*-KO mouse models, which exhibit abrogated nuclear signaling, have been created to address the roles of different TR isoforms in proper brain development and function (47, 48, 83). However, it was reported that these mice exhibit only a mild neurological phenotype compared with hypothyroid animals, indicating that the absence of T_3 binding (unliganded TR) is more harmful to the CNS than the absence of TR isoforms (46, 84) (**Table 1**). Later, *Thra*- and *Thrb*-knock-in mutant mice expressing dominant-negative TRs were generated, and it was reported that these mice were phenotypically distinct from TR-KO mice (50, 53–55). Specifically, in mice harboring the *Thrb* Δ337T mutation – a point mutation in the ligand-binding domain that prevents T_3 binding but not binding to DNA or co-factors (85) – cerebellar morphogenesis was similar to that observed in congenital hypothyroidism, presumably because TR remained constitutively bound to its co-repressors, thereby mimicking a hypothyroid state (50). Hashimoto et al. (50) demonstrated that *Thrb* Δ337T mice displayed impairments in balance and coordination, reductions in the molecular and PCLs, and decreases in the number and branching of Purkinje cells, which may account for the decreased cerebellar size observed in these mutant animals.

Therefore, functional TR-β is required for TH-dependent cerebellar development, which was further demonstrated by the phenotypes observed in *Thrb* Δ337T mutant mice, including defects in cerebellar foliation, altered laminar organization, abnormal Purkinje cell dendritogenesis, and reduced Bergmann glia fibers (51). Cerebellar foliation is characterized by the presence of 10 well-formed lobules and sub-lobules (7). In *Thrb* Δ337T

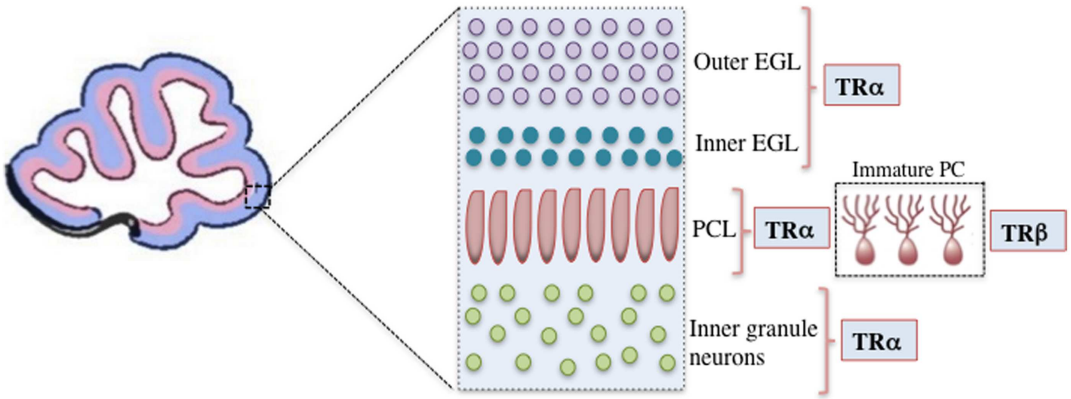

FIGURE 1 | A representation of the mouse cerebellar cortex during the initial postnatal days showing the positions of cells expressing specific TR isoforms. Only the outer EGL, inner EGL, Purkinje cell layer (PCL), and inner granule layer are shown. TRα is primarily expressed in granular cell precursors and subsequently in the transient outer and inner EGL. In the Purkinje cells, TRα is the first isoform to be detected, however, after the second postnatal week TRβ is predominantly expressed. TR, thyroid hormone receptor; EGL, external granular layer; P14, postnatal day 14; PCL, Purkinje cell layer.

homozygotes at postnatal day (PND) 21, researchers observed decreases in the molecular and granular layers as well as a failure in the subdivision of lobule VI, which is subdivided into sub-lobules VIa and VIb in wild-type and heterozygous animals. During PND 9, which is the initial period of cerebellar development, *Thrb* Δ337T mice fail to form fissures between lobules VI–VII, and lobule IX is also severely affected. During both the initial and final stages of cerebellar foliation, the *Thrb* Δ337T mutation leads to extreme defects in fissure and lobule formation (51). Unfortunately, the identification of direct target genes that are regulated by TH in the developing brain using RNA-based techniques has been problematic. However, recent studies using chromatin immunoprecipitation combined with DNA microarray analysis (ChIP on chip) identified a large number of TR-β binding sites and target genes in the developing mouse cerebellum, reinforcing the role of TR-β in mediating gene transcription through TH in this brain structure (86, 87). Chatonnet et al. introduced TR-α1 and TR-β1 into a neural cell line lacking endogenous TRs and demonstrated that the majority of the T_3 target genes analyzed were regulated by both TR-α1 and TR-β1. Nevertheless, a significant number of the analyzed genes showed strong preferences for one receptor isoform over the other (88).

In the cerebellum of mice carrying a cell-specific L400R mutation in the ligand-binding domain of TR-α1 *Thra* L400R), which prevents histone acetyltransferase recruitment and facilitates the permanent recruitment of co-repressors, there is a delay in the pattern of granule cell differentiation similar to what is observed in congenital hypothyroid animals; however, Purkinje cell arborization is not strongly affected in these mutants (55). Another study involving *Thra* L400R mice highlighted the importance of TRα-dependent signaling in postnatal brain development by showing that it promotes the secretion of neurotrophins from astrocytes and Purkinje cells and that it maintains adult brain function by limiting the proliferation of oligodendrocyte precursor cells (56). Late in their development, these mutant mice displayed a loss of axonal regenerative capacity in Purkinje cells, which is thought to play a role in the brain maturation process. These data indicate an important role for TR-α1 in mediating T_3-induced inhibition of axonal regeneration in Purkinje cells (57). In addition, it was very recently reported that the L400R mutation primarily affects the differentiation of two specific cerebellar cell populations, Purkinje cells, and Bergmann glia, which indicates that the autonomous effects of TH on these cells indirectly impact global cerebellar cortex development (58). In Purkinje cells, T_3 acts through TR-α1 to promote dendritic tree development and the secretion of neurotrophic factors, whereas in Bergmann glia, T_3 promotes the development and organization of radial fibers and the alignment of cell bodies within the PCL (58) (**Table 1**). In humans, a role for TR-α1 in brain development is supported by descriptions of patients with cognitive impairment phenotypes similar to those observed in congenital hypothyroidism who harbor primary mutations in the *THRA* gene (89, 90).

Taken together, these data suggest that TR-α and TR-β function together to mediate the processes of cerebellar ontogenesis controlled by THs. Compared with *Thrb* mutants, *Thra*-knock-in mice show more severe cerebellar defects, indicating that TR-α may play a key role in regulating the expression of target genes involved in cerebellar ontogeny (52). Other relevant mutant animal models with impaired neurological phenotypes also exist, such as *Ncoa1*-KO animals. Steroid receptor co-activator 1, which is encoded by the *Ncoa1* gene, has been shown to modulate TH activity via specific TR isoforms (91, 92). This co-activator is highly expressed in the cerebellum; thus, *Ncoa1*-KO mice exhibit cerebellar abnormalities that are similar to those observed in congenital hypothyroid mice (59).

CONCLUDING REMARKS

It has been known for decades that cerebellar development is regulated by THs. Although the molecular mechanisms through which THs impact CNS development are becoming better understood, primarily due to studies in genetic animal models, many issues remain to be addressed. Only a few T_3 targets in neural

cells have been described to date, it is important to identify additional direct target genes of THs and to determine how these genes are temporally and spatially regulated during specific neurodevelopment. Finally, the rapid non-genomic actions of THs and the role of the recently described thyronine derivatives require further analysis. Therefore, additional studies will be necessary before our model of TH activity within the developing cerebellum is complete.

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REFERENCES

1. Lazar M. Thyroid hormone action: a binding contact. *J Clin Invest* (2003) **122**:497–9. doi:10.1172/JCI19479
2. Brent GA. Mechanisms of thyroid hormone action. *J Clin Invest* (2012) **122**(9):3035–43. doi:10.1172/JCI60047
3. Anderson GW, Schoonover CM, Jones SA. Control of thyroid hormone action in the developing rat brain. *Thyroid* (2003) **13**(11):1039–56. doi:10.1089/105072503770867219
4. de Escobar GM, Obregón MJ, del Rey FE. Maternal thyroid hormones early in pregnancy and fetal brain development. *Best Pract Res Clin Endocrinol Metab* (2004) **18**(2):225–48. doi:10.1016/j.beem.2004.03.012
5. Bernal J. Thyroid hormones and brain development. *Vitam Horm* (2005) **71**:95–122. doi:10.1016/S0083-6729(05)71004-9
6. Diez D, Grijota-Martinez C, Agretti P, Marco GD, Tonacchera M, Pinchera A, et al. 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**(8):3989–4000. doi:10.1210/en.2008-0350
7. Altman J, Bayer SA. *Development of the Cerebellar System in Relation to its Evolution, Structure, and Functions*. Boca Raton: CRC Press (1997).
8. Tavano A, Grasso R, Gagliardi C, Triulzi F, Bresolin N, Fabbro F, et al. Disorders of cognitive and affective development in cerebellar malformations. *Brain* (2007) **130**(10):2646–60. doi:10.1093/brain/awm201
9. Legrand J. Effects of thyroid hormones on central nervous system development. In: Yanai J, editor. *Neurobehavioral Teratology*. Amsterdam: Elsevier (1984). p. 31–363.
10. Sillitoe RV, Joyner AI. Morphology, molecular codes, and circuitry produce the three-dimensional complexity of the cerebellum. *Annu Rev Cell Dev Biol* (2007) **23**:549–77. doi:10.1146/annurev.cellbio.23.090506.123237
11. Koibuchi N, Ikeda Y. Hormones and cerebellar development. In: Manto M, Gruol D, Schmahmann JD, Koibuchi N, editors. *Handbook of the Cerebellum and Cerebellar Disorders*. New York, NY: Springer (2013). p. 319–39.
12. Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. *Endocr Rev* (2010) **31**(2):139–70. doi:10.1210/er.2009-0007
13. Liu Y, Xia X, Fondell JD, Yen PM. Thyroid hormone-regulated target genes have distinct patterns of coactivator recruitment and histone acetylation. *Mol Endocrinol* (2006) **20**(3):483–90. doi:10.1210/me.2005-0101
14. Bradley DJ, Towle HC, Young WS. Spatial and temporal expression of alpha- and beta-thyroid hormone receptor mRNAs, including the beta-2 subtype in the mammalian nervous system. *J Neurosci* (1992) **12**:2288–302.
15. Chan S, Kachilele S, McCabe CJ, Tannahill LA, Boelaert K, Gittoes NJ, et al. Early expression of thyroid hormone deiodinases and receptors in human fetal cerebral cortex. *Brain Res Dev Brain Res* (2002) **138**:109–16. doi:10.1016/S0165-3806(02)00459-5
16. Quignodon L, Grijota-Martinez C, Compe E, Guyot R, Allioli N, Laperriere D, et al. 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. doi:10.1677/JME-06-0054
17. Davis PJ, Davis FB, Cody V. Membrane receptors mediating thyroid hormone action. *Trends Endocrinol Metab* (2005) **16**(9):429–35. doi:10.1016/j.tem.2005.09.0072
18. Farwell AP, Dubord-Tomasetti S, Pietrzykowski AZ, Leonard JL. Dynamic nongenomic actions of thyroid hormone in the developing rat brain. *Endocrinology* (2006) **147**(5):2567–74. doi:10.1210/en.2005-1272
19. Gothe S, Wang Z, Ng L, Kindblom JM, Barros AC, Ohlsson C, et al. Mice devoid of all known thyroid hormone receptors are viable but exhibit disorders of the pituitary-thyroid axis, growth, and bone maturation. *Genes Dev* (1999) **13**:1329–41. doi:10.1101/gad.13.10.1329
20. Farwell AP, Dubord-Tomasetti SA, Pietrzykowski AZ, Stachelek SJ, Leonard JL. Regulation of cerebellar neuronal migration and neurite outgrowth by thyroxine and 3,3′,5′-triiodothyronine. *Brain Res Dev Brain Res* (2005) **154**(11):121–35. doi:10.1016/j.devbrainres.2004.07.016
21. Galton VA, Schneider MJ, Clark AS, St Germain DL. Life without thyroxin to 3,5,3′-triiodothyronine conversion: studies in mice devoid of 5′-deiodinases. *Endocrinology* (2009) **150**:2958–63. doi:10.1210/en.2008-1572
22. Friesema EC, Gueters A, Biebermann H, Krude H, von Moers A, Reeser M, et al. Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet* (2004) **364**:1435–7. doi:10.1016/S0140-6736(04)17226-7
23. Heuer H, Maier MK, Iden S, Mittag J, Friesema EC, Visser TJ, et al. The monocarboxylate transporter 8 linked to human psychomotor retardation is highly expressed in thyroid hormone-sensitive neuron populations. *Endocrinology* (2005) **146**:1701–6. doi:10.1210/en.2004-1179
24. Obregón MJ, Mallol J, Pastor R, de Escobar GM, del Rey GE. L-Thyroxine and 3,5,3′-triiodo-L-thyronine in rat embryos before onset of fetal thyroid function. *Endocrinology* (1984) **144**:305–7. doi:10.1210/endo-114-1-305
25. Piosik PA, van Groenigen M, van Doorn J, Baas F, de Vijlder JJ. Effects of maternal thyroid status on thyroid hormones and growth in congenitally hypothyroid goat fetuses during the second half of gestation. *Endocrinology* (1997) **138**:5–11. doi:10.1210/endo.138.1.4843
26. Contempré B, Jauniaux E, Calvo R, Jurkovic D, Campbell S, de Escobar GM. Detection of thyroid hormones in human embryonic cavities during the first trimester of pregnancy. *J Clin Endocrinol Metab* (1993) **77**:1719–22. doi:10.1210/jcem.77.6.8263162
27. Kester MH, Martinez de Mena R, Obregon MJ, Marinkovic D, Howatson A, Visser TJ, et al. Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. *J Clin Endocrinol Metab* (2004) **89**:3117–28. doi:10.1210/jc.2003-031832
28. Boyages SC, Halpern JP. Endemic cretinism: toward a unifying hypothesis. *Thyroid* (1993) **3**:59–69. doi:10.1089/thy.1993.3.59
29. LaFranchi S. Congenital hypothyroidism: etiologies, diagnosis, and management. *Thyroid* (1999) **9**:735–40. doi:10.1089/thy.1999.9.735
30. Porterfield SP, Hendrich CE. The role of thyroid hormones in prenatal and neonatal neurological development – current perspectives. *Endocr Rev* (1993) **14**:94–106. doi:10.1210/edrv-14-1-94
31. Bernal J. Thyroid hormone receptors in brain development and function. *Nat Clin Pract Endocrinol Metab* (2007) **3**:249–59. doi:10.1038/ncpendmet0424
32. Mansouri A, Chowdhury K, Gruss P. Follicular cells of the thyroid gland require Pax8 gene function. *Nat Genet* (1998) **19**:87–90. doi:10.1038/ng0598-87
33. Poguet AL, Legrand C, Feng X, Yen PM, Meltzer P, Samarut J, et al. 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**:188–99. doi:10.1016/S0012-1606(02)00039-8
34. Friedrichsen S, Christ S, Heuer H, Schäfer MK, Mansouri A, Bauer K, et al. Regulation of iodothyronine deiodinases in the Pax8−/− mouse model of congenital hypothyroidism. *Endocrinology* (2003) **144**(3):777–84. doi:10.1210/en.2002-220715
35. Horn S, Kersseboom S, Mayer S, Muller J, Groba C, Trajkovic-Arsic M, et al. Tetrac can replace thyroid hormone during brain development in mouse mutants deficient in the thyroid hormone transporter Mct8. *Endocrinology* (2013) **154**(2):968–79. doi:10.1210/en.2012-1628
36. 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**:4036–43. doi:10.1210/en.2006-0390
37. Trajkovic M, Visser TJ, Mittag J, Horn S, Lukas J, Darras VM, et al. Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8. *J Clin Invest* (2007) **117**(3):627–35. doi:10.1172/JCI28253
38. Mayerl S, Müller J, Bauer R, Richert S, Kassmann CM, Darras VM, et al. Transporters MCT8 and OATP1C1 maintain murine brain thyroid hormone homeostasis. *J Clin Invest* (2014) **124**(5):1987–99. doi:10.1172/JCI70324
39. Mayerl S, Visser TJ, Darras VM, Horn S, Heuer H. Impact of Oatp1c1 deficiency on thyroid hormone metabolism and action in the mouse brain. *Endocrinology* (2012) **153**(3):1528–37. doi:10.1210/en.2011-1633

40. Braun D, Wirth EK, Wohlgemuth F, Reix N, Klein MO, Gruters A, et al. Aminoaciduria, but normal thyroid hormone levels and signalling, in mice lacking the amino acid and thyroid hormone transporter Slc7a8. *Biochem J* (2011) **439**(2):249–55. doi:10.1042/BJ20110759
41. Guadano-Ferraz A, Obregon MJ, St Germain DL, Bernal J. The type 2 iodothyronine deiodinase is expressed primarily in glial cells in the neonatal rat brain. *Proc Natl Acad Sci U S A* (1997) **94**:10391–6. doi:10.1073/pnas.94.19.10391
42. Galton VA, Wood ET, Germain EAS, Withrow CA, Aldrich G, Germain GMS, et al. Thyroid hormone homeostasis and action in the type 2 deiodinase-deficient rodent brain during development. *Endocrinology* (2007) **148**:3080–8. doi:10.1210/en.2006-1727
43. Hernandez A, Martinez ME, Fiering S, Galton VA, Germain DS. Type 3 deiodinase is critical for the maturation and function of the thyroid axis. *J Clin Invest* (2006) **116**(2):476–84. doi:10.1172/JCI26240
44. Hernandez A, Martinez E, Fiering S, Galton VA, St Germain DL. Type 3 deiodinase deficiency results in functional abnormalities at multiple levels of the thyroid axis. *Endocrinology* (2007) **148**:5680–7. doi:10.1210/en.2007-0652
45. Peeters RP, Hernandez A, Lily N, Michelle M, David SS, Mei P, et al. Cerebellar abnormalities in mice lacking type 3 deiodinase and partial reversal of phenotype by deletion of thyroid hormone receptor alpha1. *Endocrinology* (2013) **154**(1):550–61. doi:10.1210/en.2012-1738
46. Morte B, Manzano J, Scanlan T, Vennström B, Bernal J. Deletion of the thyroid hormone receptor α1 prevents the structural alterations of the cerebellum induced by hypothyroidism. *Proc Natl Acad Sci U S A* (2002) **99**(6):3985–9. doi:10.1073/pnas.062413299
47. Wilkstrom L, Johansson C, Salto C, Barlow C, Campo-Barros A, Baas F, et al. Abnormal heart rate and body temperature in mice lacking thyroid hormone receptor alpha 1. *EMBO J* (1998) **17**:455–61. doi:10.1093/emboj/17.2.455
48. Forrest D, Hanebuth E, Smeyne RJ, Everds N, Stewart CL, Wehner JM, et al. Recessive resistance to thyroid hormone in mice lacking thyroid hormone receptor beta: evidence for tissue-specific modulation of receptor function. *EMBO J* (1996) **15**(12):3006–15.
49. Sandhofer C, Schwartz HL, Mariash CN, Forrest D, Oppenheimer JH. Beta receptor isoforms are not essential for thyroid hormone-dependent acceleration of PCP-2 and myelin basic protein gene expression in the developing brains of neonatal mice. *Mol Cell Endocrinol* (1998) **137**:109–15. doi:10.1016/S0303-7207(98)00005-7
50. Hashimoto K, Curty FH, Borges PP, Lee CE, Abel ED, Elmquist JK, et al. An unliganded thyroid hormone receptor causes severe neurological dysfunction. *Proc Natl Acad Sci U S A* (2001) **98**:3998–4003. doi:10.1073/pnas.051454698
51. Portella A, Carvalho F, Faustino LC, Ortiga-Carvalho TM, Gomes FC. Thyroid hormone receptor beta mutation causes severe impairment of cerebellar development. *Mol Cell Neurosci* (2010) **44**(1):68–77. doi:10.1016/j.mcn.2010.02.004
52. Itoh Y, Esaki T, Kaneshige M, Suzuki H, Cook M, Sokoloff L, et al. 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**(17):9913–8. doi:10.1073/pnas.171319498
53. Tinnikov A, Nordström K, Thorén P, Kindblom JM, Malin S, Rozell B, et al. Retardation of post-natal development caused by a negatively acting thyroid hormone receptor alpha1. *EMBO J* (2002) **21**:5079–87. doi:10.1093/emboj/cdf523
54. Venero C, Guadaño-Ferraz A, Herrero AI, Nordström K, Manzano J, del Escobar GM, et al. Anxiety, memory impairment, and locomotor dysfunction caused by a mutant thyroid hormone receptor alpha1 can be ameliorated by T3 treatment. *Genes Dev* (2005) **19**(18):2152–63. doi:10.1101/gad.346105
55. 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**:2350–60. doi:10.1210/me.2007-0176
56. Picou F, Fauquier T, Chatonnet F, Flamant F. A biomodal influence of thyroid hormone on cerebellum oligodendrocyte differentiation. *Mol Endocrinol* (2012) **26**:608–18. doi:10.1210/me.2011-1316
57. Avci HX, Lebrun C, Wehrle R, Doulazmi M, Chatonnet F, Morel MP, et al. Thyroid hormone triggers the developmental loss of axonal regenerative capacity via thyroid hormone receptor α1 and Krüppel-like factor 9 in Purkinje cells. *Proc Natl Acad Sci U S A* (2012) **109**(35):14206–11. doi:10.1073/pnas.1119853109
58. Fauquier T, Chatonnet F, Picou F, Lamonerie T, Flamant F. Purkinje cells and Bergmann glia are primary targets of the TRα1 thyroid hormone receptor during mouse cerebellum postnatal development. *Development* (2014) **141**:166–75. doi:10.1242/dev.103226
59. Nishihara E, Yoshida-Komiya H, Chan CS, Liao L, Davis RL, O'Malley BW, et al. SRC-1 null mice exhibit moderate motor dysfunction and delayed development of cerebellar Purkinje cells. *J Neurosci* (2003) **23**(1):213–22.
60. Nicholson JL, Altman J. The effects of early hypo- and hyperthyroidism on the development of rat cerebellar cortex. I. Cell proliferation and differentiation. *Brain Res* (1972) **44**(1):13–23. doi:10.1016/0006-8993(72)90362-9
61. Lauder JM. The effects of early hypo- and hyperthyroidism on the development of rat cerebellar cortex. III. Kinetics of cell proliferation in the external granular layer. *Brain Res* (1977) **126**:31–51. doi:10.1016/0006-8993(77)90213-X
62. Lauder JM. Effects of early hypo- and hyperthyroidism on development of rat cerebellar cortex. IV. The parallel fibers. *Brain Res* (1978) **142**(1):25–39. doi:10.1016/0006-8993(78)90174-9
63. Heuer H, Mason CA. Thyroid hormone induces cerebellar Purkinje cell dendritic development via the thyroid hormone receptor α1. *J Neurosci* (2003) **23**(33):10604–12.
64. Martinez R, Gomes FC. Proliferation of cerebellar neurons induced by astrocytes treated with thyroid hormone is mediated by a cooperation between cell contact and soluble factors and involves the epidermal growth factor-protein kinase a pathway. *J Neurosci* (2005) **80**:341–9. doi:10.1523/jneurosci.3358-03.2004
65. Wang Y, Zhong J, Xu H, Wei W, Dong J, Yu F, et al. Perinatal iodine deficiency and hypothyroidism increase cell apoptosis and alter doublecortin and reelin protein expressions in rat cerebellum. *Arch Med Res* (2012) **43**(4):255–64. doi:10.1016/j.arcmed.2012.05.002
66. Alvarez-Dolado M, Cuadrado A, Navarro-Yubero C, Sonderegger P, Furley AJ, Bernal J, et al. Regulation of the L1 cell adhesion molecule by thyroid hormone in the developing brain. *Mol Cell Neurosci* (2000) **16**:499–514. doi:10.1006/mcne.2000.0879
67. Martinez R, Gomes FCA. Neuritogenesis induced by thyroid hormone-treated astrocytes is mediated by epidermal growth factor/mitogen-activated protein kinase-phosphatidylinositol 3-kinase pathways and involves modulation of extracellular matrix proteins. *J Biol Chem* (2002) **277**:49311–8. doi:10.1074/jbc.M209284200
68. Chakraborty G, Magagna-Poveda A, Parrat C, Umans JG, MacLusky NJ, Scharfman HE. Reduced hippocampal brain-derived neurotrophic factor (BDNF) in neonatal rats after prenatal exposure to propylthiouracil (PTU). *Endocrinology* (2012) **153**(3):1311–6. doi:10.1210/en.2011-1437
69. Martinez R, Eller C, Viana NB, Gomes FC. Thyroid hormone induces cerebellar neuronal migration and Bergmann glia differentiation through epidermal growth factor/mitogen-activated protein kinase pathway. *Eur J Neurosci* (2011) **33**(1):26–35. doi:10.1111/j.1460-9568.2010.07490
70. van der Deure WM, Peeters RP, Visser TJ. Molecular aspects of thyroid hormone transporters, including MCT8, MCT10, and OATPs, and the effects of genetic variation in these transporters. *J Mol Endocrinol* (2010) **44**(1):1–11. doi:10.1677/JME-09-0042
71. Schweizer U, Kohrle J. Function of thyroid hormone transporters in the central nervous system. *Biochim Biophys Acta* (2013) **1830**(7):3965–73. doi:10.1016/j.bbagen.2012.07.015
72. Friesema ECH, Jansen J, Heuer H, Trajkovic M, Bauer K, Visser TJ. Mechanisms of disease: psychomotor retardation and high T3 levels caused by mutations in monocarboxylate transporter 8. *Nat Clin Pract Endocrinol Metab* (2006) **2**(9):512–23. doi:10.1038/ncpendmet0262
73. Schwartz C, Stevenson RE. The MCT8 thyroid hormone transporter and Allan-Herndon-Dudley syndrome. *Best Pract Res Clin Endocrinol Metab* (2007) **21**(2):307–21. doi:10.1016/j.beem.2007.03.009
74. Friesema ECH, Visser WE, Visser TJ. Genetics and phenomics of thyroid hormone transport by MCT8. *Mol Cell Endocrinol* (2010) **322**:107–13. doi:10.1016/j.mce.2010.01.016
75. Bianco AC, Kim BW. Deiodinases: implications of the local control of thyroid hormone action. *J Clin Invest* (2006) **116**(10):2571–9. doi:10.1172/JCI29812
76. Schneider MJ, Fiering SN, Pallud SE, Parlow AF, Germain DLS, Galton VA. Targeted disruption of the type 2 selenodeiodinase gene (DIO2) results in a phenotype of pituitary resistance to T4. *Mol Endocrinol* (2001) **15**:2137–48. doi:10.1210/me.15.12.2137

77. Tata JR. The road to nuclear receptors of thyroid hormone. *Biochim Biophys Acta* (2013) **1830**(7):3860–6. doi:10.1016/j.bbagen.2012.02.017
78. 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**:3228–33. doi:10.1210/endo.137.8.8754744
79. Gil-Ibanez P, Morte B, Bernal J. Role of thyroid hormone receptor subtypes alpha and beta on gene expression in the cerebral cortex and striatum of postnatal mice. *Endocrinology* (2013) **154**:1940–7. doi:10.1210/en.2012-2189
80. Bradley DJ, Young WS, Weinberger C. Differential expression of alpha and beta thyroid hormone receptor genes in rat brain and pituitary. *Proc Natl Acad Sci U S A* (1989) **86**(18):7250–4. doi:10.1073/pnas.86.18.7250
81. Strait KA, Schwartz HL, Seybokd VS, Ling NC, Oppenheimer JH. Immunofluorescence localization of thyroid hormone receptor protein beta 1 and variant alpha 2 in selected tissues: cerebellar Purkinje cells as a model for beta 1 receptor-mediated developmental effects of thyroid hormone in brain. *Proc Natl Acad Sci U S A* (1991) **88**:3887–91. doi:10.1073/pnas.88.9.3887
82. Mellström B, Naranjo JR, Santos A, Gonzalez AM, Bernal J. Independent expression of the alpha and beta c-erbA genes in developing rat brain. *Mol Endocrinol* (1991) **5**:1339–50. doi:10.1210/mend-5-9-1339
83. Gauthier K, Chassande O, Plateroti M, Roux JP, Legrand C, Pain B, et al. Different functions for the thyroid hormone receptors TRalpha and TRbeta in the control of thyroid hormone production and post-natal development. *EMBO J* (1999) **18**:623–31. doi:10.1093/emboj/18.3.623
84. Flamant F, Samarut J. Thyroid hormone receptors: lessons from knock-out and knock-in mutant mice. *Trends Endocrinol Metab* (2003) **14**:85–90. doi:10.1016/S1043-2760(02)00043-7
85. Usala SJ, Menke JB, Watson TL, Wondisford FE, Weintraub BD, Berard J, et al. A homozygous deletion in the c-erbA beta thyroid hormone receptor gene in a patient with generalized thyroid hormone resistance: isolation and characterization of the mutant receptor. *Mol Endocrinol* (1991) **5**:327–35. doi:10.1210/mend-5-3-327
86. Dong H, Yauk CL, Rowan-Carroll A, You SH, Zoeller RT, Lambert I, et al. Identification of thyroid hormone receptor binding sites and target genes using ChIP-on-chip in developing mouse cerebellum. *PLoS One* (2009) **4**(2):e4610. doi:10.1371/journal.pone.0004610
87. Gagne R, Green JR, Dong H, Wade MG, Yauk CL. Identification of thyroid hormone receptor binding sites in developing mouse cerebellum. *BMC Genomics* (2013) **14**:341. doi:10.1186/1471-2164-14-341
88. Chatonnet F, Guyot R, Benoit G, Flamant F. Genome-wide analysis of thyroid hormone receptors shared and specific functions in neural cells. *Proc Natl Acad Sci U S A* (2013) **110**(8):E766–75. doi:10.1073/pnas.1210626110
89. Bochukova E, Schoenmakers N, Agostini M, Schoenmakers E, Rajanayagam O, Keogh JM, et al. A mutation in the thyroid hormone receptor alpha gene. *N Engl J Med* (2012) **366**:243–9. doi:10.1056/NEJMoa1110296
90. van Mullem A, van Heerebeek R, Chrysis D, Visser E, Medici M, Andrikoula M, et al. Clinical phenotype and mutant TRα1. *N Engl J Med* (2012) **366**:1451–3. doi:10.1056/NEJMc1113940
91. Weiss RE, Xu J, Ning GJ, O'Malley B, Refetoff SV. Mice deficient in the steroid receptor coactivator 1 (SRC-1) are resistant to thyroid hormone. *EMBO J* (1999) **18**:1900–4. doi:10.1093/emboj/18.7.1900
92. Mahajan MA, Samuels HH. Nuclear hormone receptor coregulator: role in hormone action, metabolism, growth, and development. *Endocr Rev* (2005) **26**(4):583–97. doi:10.1210/er.2004-0012

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Thyroid hormone signaling and adult neurogenesis in mammals

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The vital roles of thyroid hormone in multiple aspects of perinatal brain development have been known for over a century. In the last decades, the molecular mechanisms underlying effects of thyroid hormone on proliferation, differentiation, migration, synaptogenesis, and myelination in the developing nervous system have been gradually dissected. However, recent data reveal that thyroid signaling influences neuronal development throughout life, from early embryogenesis to the neurogenesis in the adult brain. This review deals with the latter phase and analyses current knowledge on the role of T_3, the active form of thyroid hormone, and its receptors in regulating neural stem cell function in the hippocampus and the subventricular zone, the two principal sites harboring neurogenesis in the adult mammalian brain. In particular, we discuss the critical roles of T_3 and TRα1 in commitment to a neuronal phenotype, a process that entails the repression of a number of genes notably that encoding the pluripotency factor, *Sox2*. Furthermore, the question of the relevance of thyroid hormone control of adult neurogenesis is considered in the context of brain aging, cognitive decline, and neurodegenerative disease.

Keywords: thyroid hormones, adult neurogenesis, brain functions, adult neural stem cells, plasticity, physiology

THYROID HORMONES AND ADULT BRAIN FUNCTION

Thyroid hormones (THs) are vital for brain organization and function throughout life. In the developing mammalian embryo prior to instigation of fetal thyroid function maternal THs are required for optimal neurogenesis (1, 2). At all life stages, but particularly during perinatal growth, T_3 is implicated in multiple processes including neurogenesis (cell cycle control and exit), synaptogenesis, migration, plasticity, and myelination (3). In adults, thyroid dysfunction correlates with neurological and behavioral disorders. Even if developmental hypothyroidism produces more deleterious, irreversible effects, adult hypothyroidism alters hippocampus function: memory impairment, anxiety, and depression-like symptoms in rodent models and humans (4, 5). In adults, the mechanisms underlying these cognitive problems are less well understood than during perinatal development. However, it is established that reduced neurogenesis, especially in the rodent hippocampus, due to either aging or stress, is associated with neurocognitive deficits such as anxiety, depression (6), and with neurodegenerative disease such as Alzheimer's (7, 8). In mammals, including humans, the subgranular zone (SGZ) of the hippocampal dentate gyrus and the subventricular zone (SVZ) represent the two main neurogenic niches. These niches produce newborn neurons from neural stem cells (NSC) throughout life and so, contribute to brain plasticity during learning, memory, and recovery from brain damage (9). Many extrinsic and intrinsic signaling factors regulate different stages of adult neurogenesis (10), with TH signaling being well known to control NSC homeostasis [see below and (11–16)]. Understanding the mechanisms underlying T_3 regulation of adult neurogenesis is crucial to develop treatments for neurocognitive disorders.

A rich literature links thyroid physiology and neurocognitive dysfunction in humans. Hypothyroidism is associated with mood instability and depression, dementia, memory impairment, and psychomotor problems (17). Most often, mood abnormalities reverse under T_4-supplementation, but can persist after long-term hypothyroidism (18). The mechanisms implicated are unknown, although T_3 levels affect serotoninergic and catecholaminergic signaling at multiple levels (19, 20), systems often targeted by antidepressants. Further, in children and adolescents (21), as well as adults (22), hypothyroidism, and reduced memory function are associated with decreased hippocampal size, suggesting that TH deficiency causes structural alterations. Thus, it is plausible that neurogenesis in rodents, and depression or other psychiatric diseases associated with hypothyroidism in humans, may be related to reduced hippocampal neurogenesis.

However, the links between cognitive deficits and neurogenesis – "the neurogenic hypothesis of depression" – are still poorly understood. Even if there is evidence for adult neurogenesis in both SVZ (23) and SGZ (24) in humans, the contribution of adult neurogenesis to human brain function, and in particular to behavioral outputs, is still questioned, a point discussed in the next section.

However, there is increasing cellular and molecular understanding of the links between TH signaling and adult neurogenesis in rodents. Adult-onset hypothyroidism reduced the number of newborn neuroblasts in the dentate gyrus (14). Furthermore, in adult hypothyroid animals displaying depressive-like behavior, neurogenesis in the dentate gyrus is reduced and dendritic arborization is impaired. TH supplementation rescues these modifications (14).

THYROID HORMONE REGULATES ADULT NEUROGENESIS

Neural stem cells in adult SGZ and SVZ slowly divide asymmetrically, giving rise to progenitors. In rodents, these highly proliferative progenitors generate neuroblasts that migrate and integrate into the pre-existing neuronal networks of the hippocampus and the olfactory bulb (OB). More recent findings highlight a third neurogenic niche within the adult rodent hypothalamus, a region regulating energy balance, food intake, and body weight (25, 26).

In humans, the functional role of adult neurogenesis is controversial (27–30). Both generation of new neuroblasts and their functional incorporation, especially in the OB, is still questioned. However, recent data showed that new neurons, probably produced from the adult SVZ, are observed in the human striatum, showing that adult human SVZ can contribute to neurogenesis at least in this region (31). A decrease of neuroblasts, expressing the neuronal precursor marker doublecortin (DCX), is observed continuously from the first year after birth, in the SVZ and SGZ (29, 30, 32, 33). However, a recent study shows that a subpopulation of hippocampal neurons is able to renew, supporting the concept that adult neurogenesis occurs in humans and could contribute to cognitive functions (24).

SVZ AND SGZ NICHES

Thyroid hormone signaling is one of the main pathways vital for adult neurogenesis. Recently, T_3 was demonstrated to exert critical roles in cell proliferation and NSC commitment toward neuroblasts in both the rodent SVZ and SGZ *in vivo* (15, 16). T_3 acts on transcription through nuclear receptors, Thyroid Hormone Receptors (TRs). In vertebrates, different isoforms derive from the *Thra* (TRα2 and TRα2) and *Thrb* (TRβ1 and TRβ2) genes. The adult hippocampus expresses TRα1, TRβ1, and β2 isoforms (16, 34), whereas only TRα1 is expressed in the adult mouse SVZ (13, 15).

T_3 regulates adult neurogenesis at different steps (proliferation, survival, differentiation, and maturation). Hypothyroidism significantly reduces progenitor proliferation in the SVZ of adult mice, whereas a short T_3 pulse restores mitotic activity to euthyroid levels (13). Similarly, using Ki67 as a proliferation marker and a BrdU incorporation protocol to measure cell proliferation limiting labeling of postmitotic cells, Montero-Pedrazuela et al. (14) demonstrated that hypothyroidism in adult rats, induces a decrease of proliferation (about 30%) in the adult SGZ that is reversed by T_4 treatment. Furthermore, hypothyroidism does not affect cell survival. In contrast, two others studies shown that hypothyroidism had no observable effect on numbers of proliferative progenitors in the adult SGZ progenitor proliferation but their survival was reduced, suggesting a role of T_3 on the postmitotic progenitors (11, 12). The reasons for these differences may reside in (i) methods for the induction of hypothyroidism (ii) and potential differences in BrdU protocols used in these studies that may or may not include postmitotic cells.

In the SGZ, TRα1 has different effects on proliferation and differentiation (16, 35). First, progenitor proliferation is unaffected by TRα1 loss ($TR\alpha1^{-/-}$ mutant) or overexpression ($TR\alpha2^{-/-}$ mutant) (35). This finding correlates with the fact that TRα1 is not expressed in progenitors within the SGZ, but is highly expressed in post-mitotic progenitors corresponding to immature neurons (35). Second, neurogenesis is increased in $TR\alpha1^{-/-}$ mice, whereas in $TR\alpha2^{-/-}$ mice (overexpression of TRα1), decreased survival reduces numbers of post-mitotic neuroblasts (35). These studies suggest that in the SGZ, T_3 acts at later steps than in the SVZ, in the post-mitotic progenitors (16, 35) (**Figure 1A**). Interestingly, the damaging effects of adult hypothyroidism on hippocampal neurogenesis are recapitulated in $TR\alpha2^{-/-}$ mice (35). The $TR\alpha2^{-/-}$ mutant, in which TRα1 is overexpressed due to the ablation of TRα2, exhibit a mixed hypo- and hyperthyroid phenotype: reduced levels of T_4/T_3 in serum, decreased growth rate and body weight, elevated heart rate suggesting that the increased TRα1 levels is associated with increased receptor effects (35, 36). In a hypothyroid context, TRα1 – in this mutant – acts as an aporeceptor due to limited T_3 availability. How the role of TRα1 aporeceptor affects adult SVZ neurogenesis is unknown. Examining this possibility should identify new TRα1 targets (of both liganded and unliganded receptors) involved in regulating adult neurogenesis.

In the SVZ, although TRα1 is absent from NSCs, it appears in proliferative Dlx2+ progenitors and is high in DCX+ neuroblasts, suggesting that TRα1 favors NSC commitment toward a neuronal phenotype [(15), **Figure 1B**]. This hypothesis is bolstered by the observation that TRα1 gain of function *in vivo* generates migrating neuroblasts entering the rostral migratory stream. Inversely, shRNA-mediated TRα1 loss of function increases numbers of SVZ NSC/progenitors. Moreover, hypothyroidism also increases NSC/progenitor populations, a situation recapitulated in mutant *TRα°/°* mice (lacking all isoforms encoded by the *TRα* locus). In hypothyroidism, NSC/progenitors are blocked during interphase (13). Thus, absence of either TRα1 or T_3 induces similar effects: increasing NSC and progenitors pools, while decreasing neuroblast numbers.

In the adult SVZ, T_3, through TRα1, acts as a neurogenic switch by repressing a key gene involved in NSC pluripotency, *Sox2* (15) (**Figure 1B**). *In vivo* loss and gain of TRα1 function approaches demonstrated that *Sox2* is directly repressed by T_3/TRα1 in progenitors. Moreover, the progenitor to neuroblast transition – governed by T_3/TRα1 – may be reinforced by T_3 repression of *CyclinD1* and *c-Myc*, involved in cell cycle progression (13, 15, 37). Thus, T_3 could regulate adult SVZ homeostasis at two levels: (i) repression of a master gene involved in NSC pluripotency and (ii) repression of cell cycle regulators.

TH SIGNALING AND HYPOTHALAMIC NEUROGENESIS?

Some authors consider that certain tanycytes (glial-like cells) in the ependymal layer are NSCs. An emerging idea is that these tanycytes are diet-responsive adult NSCs, linking food intake, body weight, and energy balance to neuronal plasticity [for reviews, see (25, 26)]. Interestingly, T_3 is a strong regulator of energy metabolism at both peripheral and central, hypothalamic, levels (15). An exciting hypothesis is that T_3 may regulate adult hypothalamic neurogenesis and thereby modulate plasticity of hypothalamic neuronal networks regulating energy balance. Many components of TH signaling are expressed in tanycytes in the rodent brain (D2, OATP1C1, MCT8, see **Figure 1C**) and in turn, tanycyte activity is critical to control of the hypothalamic/pituitary/thyroid (HPT)

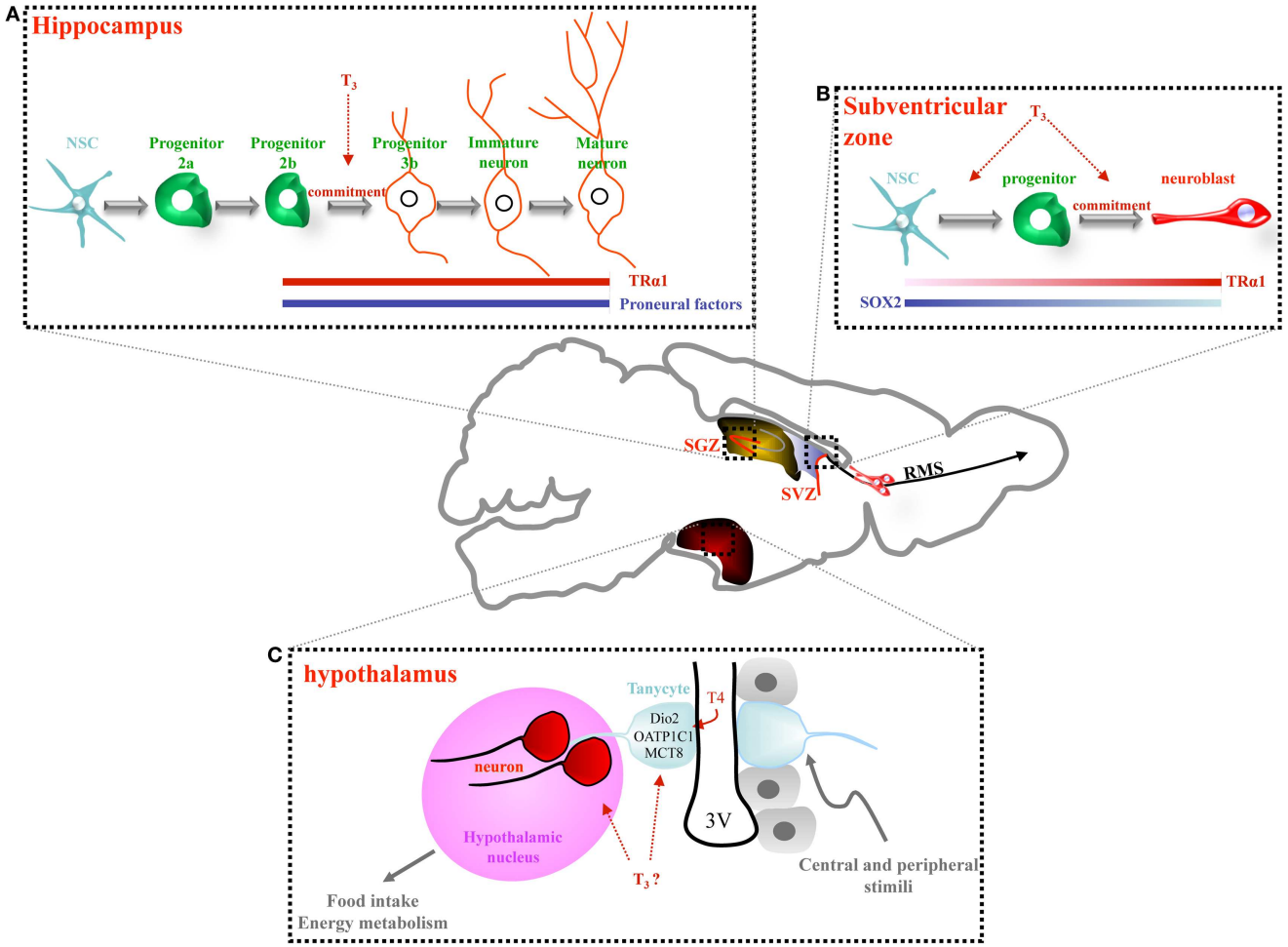

FIGURE 1 | Thyroid hormone signaling regulates adult neurogenesis in the hippocampus, the subventricular zone and, potentially, the hypothalamus. **(A)** In the hippocampal niche (SGZ), NSC gives rise proliferating progenitors (2a) and then, more committed progenitors (2b) and post-mitotic neuroblasts (type 3). Type 3 progenitors give rise to immature and mature granule neurons. A role of T_3, in concert with TRα1, has been observed in non-proliferating progenitors, from type 2b cells to mature granule cell neurons. Adult-onset hypothyroidism or TRα1 overexpression ($TR\alpha2^{-/-}$ mice) alters survival of post-mitotic neuroblasts, decreasing hippocampal neurogenesis. **(B)** In the adult SVZ, lining the lateral ventricle, three main cell types are located: NSCs that divide asymmetrically to give rise to proliferating progenitors. Progenitors divide rapidly producing neuroblasts that migrate along the rostral migratory stream (RMS) to the olfactory bulb (OB) where they differentiate into interneurons. SOX2 and TRα1 are inversely expressed within the SVZ: cells expressing high levels of TRα1 express low levels of SOX2 (neuroblasts). T_3 is involved in both progenitor proliferation and determination. TRα1 overexpression in NSC and progenitors drives progenitor commitment toward a neuronal phenotype since cells overexpressing TRα1 are found in clusters entering the RMS. **(C)** In the adult hypothalamus, the third ventricle is lined by ependymal cells (in gray) interspersed with tanycytes (in blue). Some of these tanycytes are stem cells. They possess a long process that projects into hypothalamic nuclei (in pink). Some recent data support the idea that tanycytes are able to generate new neurons that migrate into adjacent hypothalamic nuclei. Tanycytes express many key actors of the TH pathway (Dio2, OATP1C1, and MCT8) thus, facilitating TH entry into the hypothalamus. These tanycytes could be considered as an "integrative platform" relaying central and peripheral signals to adapt adult neurogenesis to food intake and energy metabolism. A key role of TH in the regulation of adult hypothalamic neurogenesis is an exciting hypothesis.

axis (38). How TH status and signaling affect adult hypothalamic neurogenesis in relation to feeding and energy balance is an important future research question.

CONTROL OF T3 AVAILABILITY DURING ADULT NEUROGENESIS

Some T_3 effects on stem cell biology can seem paradoxical, T_3 enhancing both proliferation and differentiation and exerting different actions at successive steps of neural commitment. The biological outcome of TH signaling clearly relates to cellular context, notably, chromatin state and presence of ligand, TRs, and co-factors.

One hypothesis is that adult NSCs do not integrate T_3 signaling until neural determination is underway, as TRα1 appears in neural progenitors, with the signal increasing in neuroblasts (15). In the TRα1:GFP knock-in mouse (39), expression of TRα1:GFP was not investigated closely in the SVZ. Although more data is needed on the kinetics of TR expression, a critical factor will

be T_3 availability, largely determined by deiodinases. Two deiodinases are expressed in the brain, the activating deiodinase 2 (or D2, encoded by *Dio2*) and the inactivating deiodinase 3 (or D3, encoded by *Dio3*). However, there is little published data on control of TH availability during neural determination and the little available is from *in vitro* systems. For instance, during *in vitro* neuronal differentiation of a human embryonal carcinoma stem cell line (NT2 cells derived from a teratocarcinoma), TRα1 and TRβ1 expression is down regulated, with TRα2 expression unchanged (40). T_3 treatment induced stronger upregulation of *Dio3* in NT2 precursors than in differentiated cells.

Though hypothyroid brains show reduced NSC/precursor proliferation, no clear relationship between T_3 availability and control of NSC cell cycle has yet been established. Interestingly, *Dio3* expression correlates with proliferative status in solid tumors (41). This finding fits with *in vitro* data [from Ref. (42)] where *Dio3* expression is high in early progenitors compared to human embryonic stem cells and neural progenitors. The biological significance of this finding in terms of NSC biology is hard to decipher. According to current data, local hypothyroidism favors maintenance of NSC/progenitor populations (13, 15) with T_3 being a proliferation and neurogenic factor (15, 43). Similarly, expression of *Dio3* within the imprinted *dio3-dlk1* locus is associated with stemness (44). From an evolutionary point of view, the conservation of synteny in this locus among vertebrates seems to indicate that control of TH signaling is associated with stemness.

TH CONTROL OF ADULT NEUROGENESIS IN THE AGING BRAIN

Circulating TH levels decrease as a function of age in humans (45, 46) and rodents (47). In the aging human population, both increases and decreases in circulating TSH have been observed (48–51), suggesting reduced or impaired pituitary responses in elderly people. However, higher TSH is associated with greater longevity in numerous human cohorts [see for example: (52)]. Further, neurogenesis decreases with age (53–55). THs being vital for adult neurogenesis (13), it will be interesting to address the links between these phenomena during aging.

Among the numerous genes involved in adult neurogenesis, an increase in $p16^{INKA4}$ (CDKN2a) has been causally related to neurogenic decline during aging (56). $p16^{INKA4}$ can itself be inhibited by the synergistic action of Bmi1 and c-Myc (57, 58). Direct activation of *c-Myc* by T_3 through a TRE was shown in Xenopus intestinal stem cells (59), whereas in adult SVZ T_3 directly inhibits a *c-myc* reporter construct through an identified TRE (13). Thus, a potential indirect regulation of $p16^{INKA4}$ by T_3 could differ according to species, cell populations and function of developmental context.

DECREASING CIRCULATING THs ARE ASSOCIATED WITH COGNITIVE DECLINE AND NEURODEGENERATION

Cognitive deficiency is frequently observed in the elderly humans and in aging rodents (60, 61). Marked effects are seen on learning and memory, processes that implicate neurogenesis in the dentate gyrus of the hippocampus (62, 63), a structure that diminishes with age and in many neurodegenerative pathologies (62, 64). TH treatment can improve cognitive performances in hypothyroid mice (8) and in humans (65), leading to speculation that cognitive deficiency can be causally linked to reduced TH signaling in aging. Despite declining neurogenesis with age, Yeung et al. recently demonstrated that 13-month-old mice still have the capacity to generate new neurons after a selective neuronal loss in the hippocampus, but without cognitive recovery (66). These results suggest that although some neurogenesis can still occur in aged mice, it might not be sufficient to compensate for neurodegeneration. TH facilitate repair after neurodegenerative lesions (67, 68). It is plausible that their decline is linked to decreased repair in neurodegenerative diseases of aging.

Mitochondrial biogenesis also reduces with aging (69), along with an increase in mitochondrial dysfunction (70). Thyroid signaling influences cellular metabolism and mitochondrial functions (71). Impaired thyroid signaling impacts mitochondrial respiration and hence reactive oxygen species (ROS) production, with either beneficial or damaging cellular effects (72). Since activity changes in mitochondrial respiration are linked to changes in cell proliferation rates (73), such as those occurring in the early phases of NSC differentiation, it can be postulated that mitochondrial dysfunctions impact neurogenesis, again linking reduced neurodegenerative repair capacity to decreased circulating T_3/T_4 levels. However, little is known about control of T_4/T_3 availability (deiodinase and TH transporter expression) during aging in the NSC niches, nor on the consequences of these modification for NSC metabolism, questions that it will be interesting to address.

Circadian rhythm perturbations also increase with age (74, 75). TSH (and to a lesser extent T_3) levels display circadian rhythms (76–78), as does neurogenesis (79). Moreover, circadian clock-associated genes influence neuronal differentiation of adult NSC/progenitors (80). Two major circadian rhythm regulation genes, *Bmal1* and *Clock*, are cooperatively activated by *Sirt1* and *Pgc1a,* a function that changes with age (81). In turn, SIRT1 can act as a coactivator of TRβ (82) and is implicated in neurogenesis (83). Further, *Pgc1a* is directly regulated by T_3 (84), and can itself modulate *Thra* expression (85). Some circadian clock-related genes are regulated by T_3 (86). Thus, multiple arguments converge to suggest that impairments of circadian rhythm with age can be linked to changes in thyroid signaling, thereby impacting neurogenesis.

Induction of a chronic inflammatory state has been associated with aging (87, 88), and inflammation can significantly reduce neurogenesis (89–91). Brain inflammation is characterized by macrophages and microglia producing proinflammatory cytokines (TNFα, IL-1β, and IL-6) during prolonged inflammation. These same cytokines increase in the aging brain (92), and may enhance gliogenesis at the expense of neurogenesis (93–96). TNFα activates the p38 MAP kinase (MAPKp38) that triggers IL-1β production (97). As T_3 can represses MAPKp38 activation by TNFα (98), reduced T_3 dependent repression of proinflammatory cytokines with aging could negatively impact neurogenesis.

CONCLUSION

Thyroid hormone is one of the few endocrine signals that exerts marked effects on both hippocampal and SVZ neurogenesis in adult mammalian brains. Although distinct differences are noted in expression of TRs and the consequences of their activation in these respective niches, it is well established that hypothyroidism

adversely affects both populations. Given the frequency of thyroid disorders in the general population, notably in women and during aging, it is important to consider the consequences of these disorders on the incidence and severity of psychiatric and neurodegenerative disease.

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REFERENCES

1. de Escobar GM, Obregón MJ, del Rey FE. Maternal thyroid hormones early in pregnancy and fetal brain development. *Best Pract Res Clin Endocrinol Metab* (2004) **18**:225–48. doi:10.1016/j.beem.2004.03.012
2. de Escobar GM, Obregón MJ, del Rey FE. Iodine deficiency and brain development in the first half of pregnancy. *Public Health Nutr* (2007) **10**:1554–70. doi:10.1017/S1368980007360928
3. Bernal J. Thyroid hormone receptors in brain development and function. *Nat Clin Pract Endocrinol Metab* (2007) **3**:249–59. doi:10.1038/ncpendmet0424
4. Dugbartey AT. Neurocognitive aspects of hypothyroidism. *Arch Intern Med* (1998) **158**:1413–8. doi:10.1001/archinte.158.13.1413
5. Fernández-Lamo I, Montero-Pedrazuela A, Delgado-García JM, Guadaño-Ferraz A, Gruart A. Effects of thyroid hormone replacement on associative learning and hippocampal synaptic plasticity in adult hypothyroid rats. *Eur J Neurosci* (2009) **30**(4):679–92. doi:10.1111/j.1460-9568.2009.06862.x
6. Mirescu C, Gould E. Stress and adult neurogenesis. *Hippocampus* (2006) **16**:233–8. doi:10.1002/hipo.20155
7. Breteler MM, van Duijn CM, Chandra V, Fratiglioni L, Graves AB, Heyman A, et al. Medical history and the risk of Alzheimer's disease: a collaborative re-analysis of case-control studies. EURODEM Risk Factors Research Group. *Int J Epidemiol* (1991) **20**(Suppl 2):S36–42. doi:10.1093/ije/20.Supplement_2.S36
8. Fu AL, Zhou CY, Chen X. Thyroid hormone prevents cognitive deficit in a mouse model of Alzheimer's disease. *Neuropharmacology* (2010) **58**:722–9. doi:10.1016/j.neuropharm.2009.12.020
9. Ming G-L, Song H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron* (2011) **70**:687–702. doi:10.1016/j.neuron.2011.05.001
10. Suh H, Deng W, Gage FH. Signaling in adult neurogenesis. *Annu Rev Cell Dev Biol* (2009) **25**:253–75. doi:10.1146/annurev.cellbio.042308.113256
11. Ambrogini P, Cuppini R, Ferri P, Mancini C, Ciaroni S, Voci A, et al. Thyroid hormones affect neurogenesis in the dentate gyrus of adult rat. *Neuroendocrinology* (2005) **81**:244–53. doi:10.1159/000087648
12. 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**:414–26. doi:10.1016/j.mcn.2005.03.010
13. Lemkine GF, Raj A, Alfama G, Turque N, Hassani Z, Alegria-Prévot O, et al. Adult neural stem cell cycling in vivo requires thyroid hormone and its alpha receptor. *FASEB J* (2005) **19**:863–5. doi:10.1096/fj.04-2916fje
14. Montero-Pedrazuela A, Venero C, Lavado-Autric R, Fernández-Lamo I, García-Verdugo JM, Bernal J, et al. Modulation of adult hippocampal neurogenesis by thyroid hormones: implications in depressive-like behavior. *Mol Psychiatry* (2006) **11**:361–71. doi:10.1038/sj.mp.4001802
15. López-Juárez A, Remaud S, Hassani Z, Jolivet P, Pierre Simons J, Sontag T, et al. Thyroid hormone signaling acts as a neurogenic switch by repressing Sox2 in the adult neural stem cell niche. *Cell Stem Cell* (2012) **10**:531–43. doi:10.1016/j.stem.2012.04.008
16. Kapoor R, Desouza LA, Nanavaty IN, Kernie SG, Vaidya VA. Thyroid hormone accelerates the differentiation of adult hippocampal progenitors. *J Neuroendocrinol* (2012) **24**:1259–71. doi:10.1111/j.1365-2826.2012.02329.x
17. Smith JW, Evans AT, Costall B, Smythe JW. Thyroid hormones, brain function and cognition: a brief review. *Neurosci Biobehav Rev* (2002) **26**:45–60. doi:10.1016/S0149-7634(01)00037-9
18. Joffe RT. Should thyroid replacement therapy be considered for patients with treatment-refractory depression? *J Psychiatry Neurosci* (2002) **27**:80.
19. Henley WN, Koehnle TJ. Thyroid hormones and the treatment of depression: an examination of basic hormonal actions in the mature mammalian brain. *Synapse* (1997) **27**:36–44. doi:10.1002/(SICI)1098-2396(199709)27:1<36::AID-SYN4>3.0.CO;2-E
20. Bauer M, Heinz A, Whybrow PC. Thyroid hormones, serotonin and mood: of synergy and significance in the adult brain. *Mol Psychiatry* (2002) **7**:140–56. doi:10.1038/sj.mp.4000963
21. Wheeler SM, McAndrews MP, Sheard ED, Rovet J. Visuospatial associative memory and hippocampal functioning in congenital hypothyroidism. *J Int Neuropsychol Soc* (2012) **18**:49–56. doi:10.1017/S1355617711001378
22. Cooke G, Mullally S, Correia N, O'Mara S, Gibney J. Hippocampal volume is decreased in adult-onset hypothyroidism. *Thyroid* (2013) **24**:433–40. doi:10.1089/thy.2013.0058
23. Quiñones-Hinojosa A, Sanai N, Soriano-Navarro M, Gonzalez-Perez O, Mirzadeh Z, Gil-Perotin S, et al. Cellular composition and cytoarchitecture of the adult human subventricular zone: a niche of neural stem cells. *J Comp Neurol* (2006) **494**:415–34. doi:10.1002/cne.20798
24. Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, et al. Dynamics of hippocampal neurogenesis in adult humans. *Cell* (2013) **153**:1219–27. doi:10.1016/j.cell.2013.05.002
25. Bolborea M, Dale N. Hypothalamic tanycytes: potential roles in the control of feeding and energy balance. *Trends Neurosci* (2013) **36**:91–100. doi:10.1016/j.tins.2012.12.008
26. Cheng M-F. Hypothalamic neurogenesis in the adult brain. *Front Neuroendocrinol* (2013) **34**:167–78. doi:10.1016/j.yfrne.2013.05.001
27. Eriksson PS, Perfilieva E, Björk-Eriksson T, Alborn AM, Nordborg C, Peterson DA, et al. Neurogenesis in the adult human hippocampus. *Nat Med* (1998) **4**(11):1313–7. doi:10.1038/3305
28. Arellano JI, Rakic P. Neuroscience: gone with the wean. *Nature* (2011) **478**:333–4. doi:10.1038/478333a
29. Sanai N, Nguyen T, Ihrie RA, Mirzadeh Z, Tsai H-H, Wong M, et al. Corridors of migrating neurons in the human brain and their decline during infancy. *Nature* (2011) **478**:382–6. doi:10.1038/nature10487
30. Wang X, Lui JH, Kriegstein AR. Orienting fate: spatial regulation of neurogenic divisions. *Neuron* (2011) **72**:191–3. doi:10.1016/j.neuron.2011.10.003
31. Ernst A, Alkass K, Bernard S, Salehpour M, Perl S, Tisdale J, et al. Neurogenesis in the striatum of the adult human brain. *Cell* (2014) **156**(5):1072–83. doi:10.1016/j.cell.2014.01.044
32. Göritz C, Frisén J. Neural stem cells and neurogenesis in the adult. *Cell Stem Cell* (2012) **10**:657–9. doi:10.1016/j.stem.2012.04.005
33. Knoth R, Singec I, Ditter M, Pantazis G, Capetian P, Meyer RP, et al. Murine features of neurogenesis in the human hippocampus across the lifespan from 0 to 100 years. *PLoS One* (2010) **5**:e8809. doi:10.1371/journal.pone.0008809
34. Kapoor R, Ghosh H, Nordstrom K, Vennstrom B, Vaidya VA. Loss of thyroid hormone receptor β is associated with increased progenitor proliferation and NeuroD positive cell number in the adult hippocampus. *Neurosci Lett* (2011) **487**:199–203. doi:10.1016/j.neulet.2010.10.022
35. Kapoor R, van Hogerlinden M, Wallis K, Ghosh H, Nordstrom K, Vennstrom B, et al. Unliganded thyroid hormone receptor alpha1 impairs adult hippocampal neurogenesis. *FASEB J* (2010) **24**:4793–805. doi:10.1096/fj.10-161802
36. Saltó C, Kindblom JM, Johansson C, Wang Z, Gullberg H, Nordström K, et al. Ablation of TRalpha2 and a concomitant overexpression of Alpha1 yields a mixed hypo- and hyperthyroid phenotype in mice. *Mol Endocrinol* (2001) **15**(12):2115–28. doi:10.1210/mend.15.12.0750
37. Hassani Z, François J-C, Alfama G, Dubois GM, Paris M, Giovannangeli C, et al. A hybrid CMV-H1 construct improves efficiency of PEI-delivered shRNA in the mouse brain. *Nucleic Acids Res* (2007) **35**:e65. doi:10.1093/nar/gkm152
38. Fekete C, Lechan RM. Central regulation of hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions. *Endocr Rev* (2013) **35**:159–94. doi:10.1210/er.2013-1087
39. Wallis K, Susi D, van Hogerlinden M, Nordström K, Mittag J, Vennström B. The thyroid hormone receptor Alpha1 protein is expressed in embryonic postmitotic neurons and persists in most adult neurons. *Mol Endocrinol* (2010) **24**(10):1904–16. doi:10.1210/me.2010-0175
40. Chan S, McCabe CJ, Visser TJ, Franklyn JA, Kilby MD. Thyroid hormone responsiveness in N-Tera-2 cells. *J Endocrinol* (2003) **178**:159–67. doi:10.1677/joe.0.1780159
41. Dentice M, Marsili A, Ambrosio R, Guardiola O, Sibilio A, Paik J-H, et al. The FoxO3/type 2 deiodinase pathway is required for normal mouse myogenesis

and muscle regeneration. *J Clin Invest* (2010) **120**:4021–30. doi:10.1172/JCI43670
42. Wu JQ, Habegger L, Noisa P, Szekely A, Qiu C, Hutchison S, et al. Dynamic transcriptomes during neural differentiation of human embryonic stem cells revealed by short, long, and paired-end sequencing. *Proc Natl Acad Sci U S A* (2010) **107**:5254–9. doi:10.1073/pnas.0914114107
43. Chen C, Zhou Z, Zhong M, Zhang Y, Li M, Zhang L, et al. Thyroid hormone promotes neuronal differentiation of embryonic neural stem cells by inhibiting STAT3 signaling through TRα1. *Stem Cells Dev* (2012) **21**:2667–81. doi:10.1089/scd.2012.0023
44. Liu L, Luo G-Z, Yang W, Zhao X, Zheng Q, Lv Z, et al. Activation of the imprinted Dlk1-Dio3 region correlates with pluripotency levels of mouse stem cells. *J Biol Chem* (2010) **285**:19483–90. doi:10.1074/jbc.M110.131995
45. Chakraborti S, Chakraborti T, Mandal M, Das S, Batabyal SK. Hypothalamic-pituitary-thyroid axis status of humans during development of ageing process. *Clin Chim Acta* (1999) **288**:137–45. doi:10.1016/S0009-8981(99)00061-3
46. Hertoghe T. The "multiple hormone deficiency" theory of aging: is human senescence caused mainly by multiple hormone deficiencies? *Ann N Y Acad Sci* (2005) **1057**:448–65. doi:10.1196/annals.1322.035
47. Cao L, Wang F, Yang Q-G, Jiang W, Wang C, Chen Y-P, et al. Reduced thyroid hormones with increased hippocampal SNAP-25 and Munc18-1 might involve cognitive impairment during aging. *Behav Brain Res* (2012) **229**:131–7. doi:10.1016/j.bbr.2012.01.014
48. Boucai L, Surks MI. Reference limits of serum TSH and free T4 are significantly influenced by race and age in an urban outpatient medical practice. *Clin Endocrinol (Oxf)* (2009) **70**:788–93. doi:10.1111/j.1365-2265.2008.03390.x
49. Hadlow NC, Rothacker KM, Wardrop R, Brown SJ, Lim EM, Walsh JP. The relationship between TSH and free T4 in a large population is complex and non-linear and differs by age and sex. *J Clin Endocrinol Metab* (2013) **98**:2936–43. doi:10.1210/jc.2012-4223
50. Surks MI, Hollowell JG. Age-specific distribution of serum thyrotropin and antithyroid antibodies in the US population: implications for the prevalence of subclinical hypothyroidism. *J Clin Endocrinol Metab* (2007) **92**:4575–82. doi:10.1210/jc.2007-1499
51. Peeters RP. Thyroid hormones and aging. *Horm Athens Greece* (2008) **7**:28–35. doi:10.14310/horm.2002.1111035
52. Rozing MP, Houwing-Duistermaat JJ, Slagboom PE, Beekman M, Frölich M, de Craen AJM, et al. Familial longevity is associated with decreased thyroid function. *J Clin Endocrinol Metab* (2010) **95**:4979–84. doi:10.1210/jc.2010-0875
53. Enwere E, Shingo T, Gregg C, Fujikawa H, Ohta S, Weiss S. Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination. *J Neurosci* (2004) **24**:8354–65. doi:10.1523/JNEUROSCI.2751-04.2004
54. Gould E, Reeves AJ, Fallah M, Tanapat P, Gross CG, Fuchs E. Hippocampal neurogenesis in adult Old World primates. *Proc Natl Acad Sci U S A* (1999) **96**:5263–7. doi:10.1073/pnas.96.9.5263
55. Kuhn HG, Dickinson-Anson H, Gage FH. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J Neurosci* (1996) **16**:2027–33.
56. Molofsky AV, Slutsky SG, Joseph NM, He S, Pardal R, Krishnamurthy J, et al. Increasing p16INK4a expression decreases forebrain progenitors and neurogenesis during ageing. *Nature* (2006) **443**:448–52. doi:10.1038/nature05091
57. Guney I, Wu S, Sedivy JM. Reduced c-Myc signaling triggers telomere-independent senescence by regulating Bmi-1 and p16(INK4a). *Proc Natl Acad Sci U S A* (2006) **103**:3645–50. doi:10.1073/pnas.0600069103
58. Jacobs JJ, Kieboom K, Marino S, DePinho RA, van Lohuizen M. The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. *Nature* (1999) **397**:164–8. doi:10.1038/16476
59. Fujimoto K, Matsuura K, Hu-Wang E, Lu R, Shi Y-B. Thyroid hormone activates protein arginine methyltransferase 1 expression by directly inducing c-Myc transcription during Xenopus intestinal stem cell development. *J Biol Chem* (2012) **287**:10039–50. doi:10.1074/jbc.M111.335661
60. Bach ME, Barad M, Son H, Zhuo M, Lu YF, Shih R, et al. Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signaling pathway. *Proc Natl Acad Sci U S A* (1999) **96**:5280–5. doi:10.1073/pnas.96.9.5280
61. Cao L, Jiang W, Wang F, Yang Q-G, Wang C, Chen Y-P, et al. The reduced serum free triiodothyronine and increased dorsal hippocampal SNAP-25 and Munc18-1 had existed in middle-aged CD-1 mice with mild spatial cognitive impairment. *Brain Res* (2013) **1540**:9–20. doi:10.1016/j.brainres.2013.09.034
62. Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ. Learning enhances adult neurogenesis in the hippocampal formation. *Nat Neurosci* (1999) **2**:260–5. doi:10.1038/6365
63. van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus. *Nature* (2002) **415**:1030–4. doi:10.1038/4151030a
64. Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. *Cell* (2008) **132**:645–60. doi:10.1016/j.cell.2008.01.033
65. Kramer CK, von Mühlen D, Kritz-Silverstein D, Barrett-Connor E. Treated hypothyroidism, cognitive function, and depressed mood in old age: the Rancho Bernardo Study. *Eur J Endocrinol* (2009) **161**:917–21. doi:10.1530/EJE-09-0606
66. Yeung ST, Myczek K, Kang AP, Chabrier MA, Baglietto-Vargas D, Laferla FM. Impact of hippocampal neuronal ablation on neurogenesis and cognition in the aged brain. *Neuroscience* (2014) **259**:214–22. doi:10.1016/j.neuroscience.2013.11.054
67. Calzà L, Fernandez M, Giardino L. Cellular approaches to central nervous system remyelination stimulation: thyroid hormone to promote myelin repair via endogenous stem and precursor cells. *J Mol Endocrinol* (2010) **44**:13–23. doi:10.1677/JME-09-0067
68. Lin H-Y, Davis FB, Luidens MK, Mousa SA, Cao JH, Zhou M, et al. Molecular basis for certain neuroprotective effects of thyroid hormone. *Front Mol Neurosci* (2011) **4**:29. doi:10.3389/fnmol.2011.00029
69. Derbré F, Gomez-Cabrera MC, Nascimento AL, Sanchis-Gomar F, Martinez-Bello VE, Tresguerres JAF, et al. Age associated low mitochondrial biogenesis may be explained by lack of response of PGC-1α to exercise training. *Age Dordr* (2012) **34**:669–79. doi:10.1007/s11357-011-9264-y
70. Park CB, Larsson N-G. Mitochondrial DNA mutations in disease and aging. *J Cell Biol* (2011) **193**:809–18. doi:10.1083/jcb.201010024
71. Weitzel JM, Iwen KA. Coordination of mitochondrial biogenesis by thyroid hormone. *Mol Cell Endocrinol* (2011) **342**:1–7. doi:10.1016/j.mce.2011.05.009
72. Long YC, Tan TMC, Inoue T, Tang BL. The biochemistry and cell biology of aging: metabolic regulation through mitochondrial signaling. *Am J Physiol Endocrinol Metab* (2014) **306**:E581–91. doi:10.1152/ajpendo.00665.2013
73. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* (2009) **324**:1029–33. doi:10.1126/science.1160809
74. Campos Costa I, Nogueira Carvalho H, Fernandes L. Aging, circadian rhythms and depressive disorders: a review. *Am J Neurodegener Dis* (2013) **2**:228–46.
75. Froy O. Circadian rhythms, aging, and life span in mammals. *Physiology (Bethesda)* (2011) **26**:225–35. doi:10.1152/physiol.00012.2011
76. Bitman J, Kahl S, Wood DL, Lefcourt AM. Circadian and ultradian rhythms of plasma thyroid hormone concentrations in lactating dairy cows. *Am J Physiol* (1994) **266**:R1797–803.
77. Gancedo B, Alonso-Gómez AL, de Pedro N, Delgado MJ, Alonso-Bedate M. Changes in thyroid hormone concentrations and total contents through ontogeny in three anuran species: evidence for daily cycles. *Gen Comp Endocrinol* (1997) **107**:240–50. doi:10.1006/gcen.1997.6922
78. Morris CJ, Aeschbach D, Scheer FAJL. Circadian system, sleep and endocrinology. *Mol Cell Endocrinol* (2012) **349**:91–104. doi:10.1016/j.mce.2011.09.003
79. Bouchard-Cannon P, Mendoza-Viveros L, Yuen A, Kærn M, Cheng H-YM. The circadian molecular clock regulates adult hippocampal neurogenesis by controlling the timing of cell-cycle entry and exit. *Cell Rep* (2013) **5**:961–73. doi:10.1016/j.celrep.2013.10.037
80. Kimiwada T, Sakurai M, Ohashi H, Aoki S, Tominaga T, Wada K. Clock genes regulate neurogenic transcription factors, including NeuroD1, and the neuronal differentiation of adult neural stem/progenitor cells. *Neurochem Int* (2009) **54**:277–85. doi:10.1016/j.neuint.2008.12.005
81. Chang H-C, Guarente L. SIRT1 mediates central circadian control in the SCN by a mechanism that decays with aging. *Cell* (2013) **153**:1448–60. doi:10.1016/j.cell.2013.05.027
82. Suh JH, Sieglaff DH, Zhang A, Xia X, Cvoro A, Winnier GE, et al. SIRT1 is a direct coactivator of thyroid hormone receptor β1 with gene-specific actions. *PLoS One* (2013) **8**:e70097. doi:10.1371/journal.pone.0070097

83. Rafalski VA, Ho PP, Brett JO, Ucar D, Dugas JC, Pollina EA, et al. Expansion of oligodendrocyte progenitor cells following SIRT1 inactivation in the adult brain. *Nat Cell Biol* (2013) **15**:614–24. doi:10.1038/ncb2735
84. Wulf A, Harneit A, Kröger M, Kebenko M, Wetzel MG, Weitzel JM. T3-mediated expression of PGC-1alpha via a far upstream located thyroid hormone response element. *Mol Cell Endocrinol* (2008) **287**:90–5. doi:10.1016/j.mce.2008.01.017
85. Thijssen-Timmer DC, Schiphorst MP-T, Kwakkel J, Emter R, Kralli A, Wiersinga WM, et al. PGC-1alpha regulates the isoform mRNA ratio of the alternatively spliced thyroid hormone receptor alpha transcript. *J Mol Endocrinol* (2006) **37**:251–7. doi:10.1677/jme.1.01914
86. Diez D, Grijota-Martinez C, Agretti P, De Marco G, Tonacchera M, Pinchera A, et al. 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**(8):3989–4000. doi:10.1210/en.2008-0350
87. Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, et al. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech Ageing Dev* (2007) **128**:92–105. doi:10.1016/j.mad.2006.11.016
88. Strohacker K, Breslin WL, Carpenter KC, McFarlin BK. Aged mice have increased inflammatory monocyte concentration and altered expression of cell-surface functional receptors. *J Biosci* (2012) **37**:55–62. doi:10.1007/s12038-011-9169-z
89. Butovsky O, Ziv Y, Schwartz A, Landa G, Talpalar AE, Pluchino S, et al. Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. *Mol Cell Neurosci* (2006) **31**:149–60. doi:10.1016/j.mcn.2005.10.006
90. Ekdahl CT, Claasen J-H, Bonde S, Kokaia Z, Lindvall O. Inflammation is detrimental for neurogenesis in adult brain. *Proc Natl Acad Sci U S A* (2003) **100**:13632–7. doi:10.1073/pnas.2234031100
91. Monje ML, Toda H, Palmer TD. Inflammatory blockade restores adult hippocampal neurogenesis. *Science* (2003) **302**:1760–5. doi:10.1126/science.1088417
92. Terao A, Apte-Deshpande A, Dousman L, Morairty S, Eynon BP, Kilduff TS, et al. Immune response gene expression increases in the aging murine hippocampus. *J Neuroimmunol* (2002) **132**:99–112. doi:10.1016/S0165-5728(02)00317-X
93. Koo JW, Duman RS. IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc Natl Acad Sci U S A* (2008) **105**:751–6. doi:10.1073/pnas.0708092105
94. Lan X, Chen Q, Wang Y, Jia B, Sun L, Zheng J, et al. TNF-α affects human cortical neural progenitor cell differentiation through the autocrine secretion of leukemia inhibitory factor. *PLoS One* (2012) **7**:e50783. doi:10.1371/journal.pone.0050783
95. Vallières L, Campbell IL, Gage FH, Sawchenko PE. Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. *J Neurosci* (2002) **22**:486–92.
96. Zunszain PA, Anacker C, Cattaneo A, Choudhury S, Musaelyan K, Myint AM, et al. Interleukin-1β: a new regulator of the kynurenine pathway affecting human hippocampal neurogenesis. *Neuropsychopharmacology* (2012) **37**:939–49. doi:10.1038/npp.2011.277
97. Kim SH, Smith CJ, Van Eldik LJ. Importance of MAPK pathways for microglial pro-inflammatory cytokine IL-1 beta production. *Neurobiol Aging* (2004) **25**:431–9. doi:10.1016/S0197-4580(03)00126-X
98. Lasa M, Gil-Araujo B, Palafox M, Aranda A. Thyroid hormone antagonizes tumor necrosis factor-alpha signaling in pituitary cells through the induction of dual specificity phosphatase 1. *Mol Endocrinol* (2010) **24**:412–22. doi:10.1210/me.2009-0298

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Thyroid hormones, T3 and T4, in the brain

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Thyroid hormones (THs) are essential for fetal and post-natal nervous system development and also play an important role in the maintenance of adult brain function. Of the two major THs, T_4 (3,5,3′,5′-tetraiodo-l-thyronine) is classically viewed as an pro-hormone that must be converted to T_3 (3,5,3′-tri-iodo-l-thyronine) via tissue-level deiodinases for biological activity. THs primarily mediate their effects by binding to thyroid hormone receptor (TR) isoforms, predominantly TRα1 and TRβ1, which are expressed in different tissues and exhibit distinctive roles in endocrinology. Notably, the ability to respond to T_4 and to T_3 differs for the two TR isoforms, with TRα1 generally more responsive to T_4 than TRβ1. TRα1 is also the most abundantly expressed TR isoform in the brain, encompassing 70–80% of all TR expression in this tissue. Conversion of T_4 into T_3 via deiodinase 2 in astrocytes has been classically viewed as critical for generating local T_3 for neurons. However, deiodinase-deficient mice do not exhibit obvious defectives in brain development or function. Considering that TRα1 is well-established as the predominant isoform in brain, and that TRα1 responds to both T_3 and T_4, we suggest T_4 may play a more active role in brain physiology than has been previously accepted.

Keywords: T4 thyronine, T3 thyronine, thyroid hormone receptor, brain, coregulator, deiodinase 2

INTRODUCTION

Thyroid hormones (THs) are synthesized by the thyroid gland and are critical regulatory molecules with important roles in vertebrate physiology and development, including fetal and post-natal nervous system development and the maintenance of adult brain function (1, 2). The TH requirement for development is most apparent in the central nervous system (CNS) where severe TH deficiency in fetal and neonatal periods results in cretinism, a disease characterized by mental retardation, deafness, and ataxia; these consequences are irreversible if not treated soon after birth (3–5). Additionally, untreated hypothyroidism in the adult is associated with severe intellectual defects, abnormal balance and defects in fine motor skills, spasticity, and deafness (6). Correcting TH deficiencies is critical for normal brain development and function.

Thyroid hormones mediate CNS effects primarily through thyroid hormone receptors (TRs), members of the nuclear hormone receptor family (4, 7, 8). TRs bind to the DNA regulatory regions of target genes to activate or repress transcription through interactions with accessory proteins known as coregulators. There are two major THs, which bind to and activate TRs: T_3 (3,5,3′-triiodo-L-thyronine) and T_4 (3,5,3′,5′-tetraiodo-L-thyronine, also known as thyroxine). T_4 differs from T_3 by an additional iodine located at the 5′-position of the first thyroxine ring. T_3 has been assumed to be the active form of TH, as T_3 binds to TRs with a greater affinity than T_4. In this model, T_4 is thought to simply act as a pro-hormone, existing only to be circulated in the serum and converted at the tissue-level to T_3 through an enzymatic reaction involving the removal of the 5′-iodine atom from T_4 by local deiodinases (9, 10). Nonetheless, it is notable that most of the TH produced under normal conditions in the thyroid is secreted in the form of T_4 and steady-state serum concentrations of T_4 are many fold greater than those of T_3 (11–14). Notably, iodine intake is important for the maintenance of both of these TH levels in circulation. In fact, during gestation and lactation in females, double the normal iodine intake is required to maintain adequate T_3 and T_4 in circulation to ensure normal fetal development (15, 16). Under conditions of low iodine intake, the serum T_3/T_4 ratio is somewhat increased reflecting the reduced abundance of iodine atoms (16). Although the ready availability of dietary iodized salt has largely eliminated these iodine deficiencies for school children in most developed countries today, these advances are often not adequate for pregnant and lactating women (17).

Indeed the primary TH crossing the adult blood–brain barrier (BBB) is believed to be T_4; therefore, the adult brain may have access to sufficiently high levels of T_4 to allow for direct binding to and transcriptional activation of TRs (18, 19). In fact, we know that both T_4 and T_3 binding by TRs lead to very similar structural changes in the receptor (12). Several reports have also shown that T_4 exhibits non-genomic effects by interacting with integrin cell membrane receptors (20). These studies suggest that T_4 may exhibit a greater role in physiology than merely acting as a pro-hormone. Therefore, the precise role of T_4 as a pro-hormone and whether T_4 might function directly as an active hormone in the CNS, remain incompletely answered questions.

T_4 SYNTHESIS, TRANSPORT, AND AVAILABILITY IN THE BRAIN

Determining the effective cellular concentrations of T_4 and T_3 in the brain, or in any tissue, is difficult due to the complexities of TH synthesis, transport, and regulation. Vertebrates have developed multiple mechanisms to ensure delivery of appropriate levels of TH to peripheral tissues such as the brain. These include regulation of secretion of THs from the thyroid into serum (21, 22),

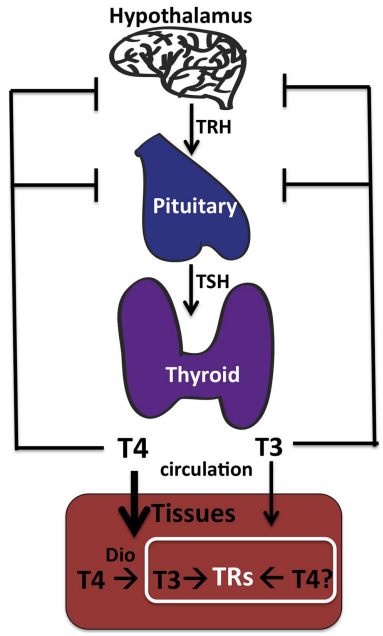

FIGURE 1 | Thyroid hormone synthesis. The thyroid gland makes both T_4 and T_3, although T_4 predominates. The hypothalamus senses low TH in the circulation and responds by stimulating synthesis and secretion of TRH (thyroid releasing hormone), which in turn circulates and stimulates synthesis and secretion of TSH (thyroid stimulating hormone) by the pituitary. Circulating TSH then increases T_4 and T_3 production by the thyroid and ultimately in the circulation. Tissue-specific deiodinases ("DIO") are expressed in peripheral tissues such as brain astrocytes to increase local concentrations of T_3 from circulating T_4. However, we propose that T_4 may also act directly on TRs to regulate gene transcription in neurons in the absence of deiodinase 2 conversion to T_3.

control of free versus bound levels of THs determined by reversible binding to serum-binding proteins (22), cell-specific expression of TH cell membrane transporters (23, 24), and finally intracellular deiodination of T_4 to form T_3 [(22, 25); **Figure 1**].

Transplacental TH transfer from maternal to fetal circulation is particularly important in vertebrate CNS development [reviewed by Ref. (26)] to ensure appropriate levels of TH are available to the fetus throughout development (16). Throughout the first trimester when TH levels are solely obtained through maternal transfer, free T_4 levels are high in the fetus, similar to levels of biologically active T_4 in adults, whereas fetal concentrations of T_3 are at least 10× lower than T_4 (16). Notably, T_3 levels in the fetal cerebral cortex increase somewhat between 12 and 20 weeks PMA (post-menstrual age) when placental deiodinase 2 levels increase (see below), although maternal serum levels of T_3 are still low. Both T_4 and T_3 in the fetus continue to be transferred from maternal origins through the placenta until half-way through pregnancy when endogenous THs are produced by the fetal thyroid. However, because fetal T_4 synthesis is elevated over that of T_3 for several weeks at this time, it is possible that an additional window in development exists where fetal circulating T_4 is quite high and may act as an active hormone with TRs (16).

Outer ring 5′-monodeiodination via cell-specific deiodinases converts a small fraction of the normal serum T_4 pool to T_3 (10, 22). Deiodinase 2 is the primary enzyme responsible for intracellular conversion of T_4 into T_3 in most local tissues including brain, whereas deiodinase 1 is found primarily in the liver (25, 27). Deiodinase 2 is only expressed in selected cell types within the CNS: astrocytes and tanycytes. These are both glial cell-derived and are located in the hypothalamus (28–30). The other deiodinase enzyme expressed in the CNS is deiodinase 3, selectively expressed in neurons. Deiodinase 3 inactivates both T_4 and T_3 by inner ring deiodination to rT_3 and T_2 so as to down-regulate local TH concentrations and protect the neuron from supraphysiological levels of TH. Currently it is believed that astrocytes generate active T_3 from circulating pro-hormone, T_4, whereas neurons degrade both T_4 and T_3 to inactive rT3 and T2, respectively, and thereby regulate local TH availability within the brain. When levels of TH are low, deiodinase 2 levels in brain increase and contrastingly when there are high levels of TH, deiodinase 3 levels increase (19, 30, 31). This balancing act protects the brain from the detrimental effects of hyper- or hypothyroidism.

T_3 concentrations equilibrate rapidly in peripheral tissues such as the liver and kidney but appear to take longer to equilibrate in the brain. In general, TH concentrations in the CNS are approximately 20% that of serum concentrations (32); this is likely due to the added complexity of TH transport across the BBB, which is comprised of the endothelial cells of brain capillaries surrounded by astrocyte end feet. To enter the brain, the THs cross the BBB of the choroid plexus via the MCT8 or OATP1C1 TH transporters. T_4 is thought to predominately enter the CNS in preference to T_3 as the majority of BBB TH transporters exhibit greater affinities for T_4 transport [(19, 33); **Figure 2**]. As mentioned above, after T_4 is taken up into astrocytes likely by OATP1C1, deiodinase 2 can in turn convert it locally to T_3. Finally, the astrocyte-generated T_3 can enter neuronal cells via the MCT8 transporter to bind and activate TRs. Therefore, it is intriguing that the T_4-activating deiodinase is not expressed in the neurons themselves, where the relevant TRs are located, but in the astrocytes. T_4 and/or T_3 also enter the CNS directly via gaps in the end feet of the astrocytes, which do not completely cover the capillaries in contact with the interstitial spinal fluid (34).

DIFFERENT TR ISOFORMS DIFFER IN THEIR ABILITY TO BIND TO T_4

Thyroid hormones bind TRs, ligand-regulated transcription factors, which bind to specific target DNA sequences and repress or activate target genes through the recruitment and release of accessory proteins. TRs contact their DNA-binding elements as protein dimers, heterodimerizing with another member of the nuclear receptor family, RXRs (primarily Retinoid X Receptors), or homodimerizing with themselves (35–39). TRs exhibit bimodal regulation, typically binding corepressors to repress transcription of target genes in the absence of TH, but releasing corepressors and recruiting coactivators to activate transcription of these "positive response" target genes in the presence of TH (40, 41). These corepressor and coactivator proteins alter the chromatin template or interact with the general transcription machinery to produce the appropriate transcriptional outputs. However, many TR target

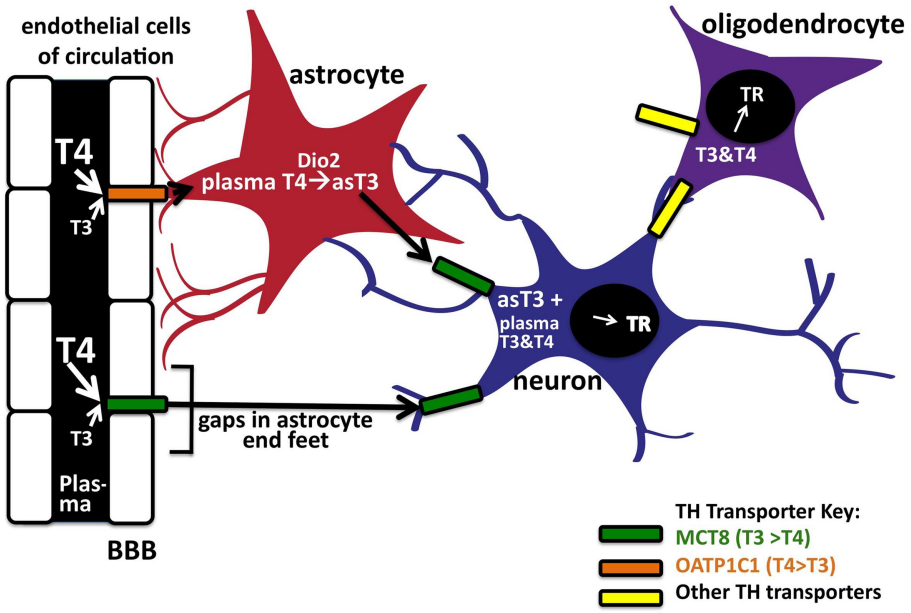

FIGURE 2 | Entry of TH into brain via the blood–brain barrier. TH can enter neurons by two pathways. The first is by crossing the endothelial cells of the blood–brain barrier (BBB) by the OAT1P1C transporter to enter astrocyte end feet (in red). After entering astrocytes, T_4 can be converted into T_3 via deiodinase 2, to enter the neuron (in blue) by the MCT8 transporter. Circulating T_4 and T_3 may also enter neurons (and astrocytes) directly via these transporters through gaps in the astrocyte end feet. Oligodendrocytes (purple), which express TRs, are also known TH cell targets in the CNS. There is also evidence of as-yet unknown TH transporters in the brain; the TH transporters, and their known preferences for T_4 or T_3, are indicated in color codes on the right.

genes display the opposite properties in that they are expressed in the absence of TH and are repressed in the presence of TH; the molecular mechanisms involved in this "negative response" is not well-understood.

Thyroid hormone receptors are encoded by two distinct genetic loci, denoted THRA and THRB, which are each expressed as alternatively spliced mRNAs to create additional receptor diversity [reviewed in Ref. (42)]. Two of the major TR isoforms are referred to as TRα1 and TRβ1; both bind TH and yet exhibit distinct biological roles [reviewed in Ref. (43)]. TRα1 is expressed early in embryonic development and then widely in adults whereas TRβ1 is expressed later in embryonic development and exhibits a more restricted tissue-expression pattern in adults (31, 44–49). Genetic disruption in mice of TRα1 or TRβ1 indicates that these isoforms have somewhat overlapping, yet distinct roles in normal physiology (45–47, 49, 50).

These two different TR isoforms differ in their ability to respond to T_4, with TRα1 generally exhibiting a much stronger response to T_4 than TRβ1. We suggest that different cell types may modulate their relative ability to respond to T_4 versus T_3 by altering the relative abundance of different coactivators and corepressors that have distinct responses to T_4 and T_3, raising the possibility that T_4 may be able to function as a direct-acting hormone agonist with TRα1 (Amy C. Schroeder and Martin L. Privalsky, unpublished observations).

TRα1 EXPRESSION IN THE BRAIN

Notably, TRα1 encompasses 70–80% of all TR expression in the adult vertebrate brain (2) and TRα1 is present in nearly all neurons (51). Intriguingly, TRα1 is also the predominating TR isoform early in fetal brain development (detected by 8.1 weeks and increasing until 13.9 weeks post-menstrual age). Critical roles in CNS development are known to be mediated by TRα1 including TH-dependent oligodendrocyte differentiation (52). If TRα1 is inactivated, the number of mature oligodendrocytes after T_3 treatment is decreased (52). The commitment of these cells as oligodendrocytes is therefore believed to be linked to cell-specific TRα1 expression while the availability of TH regulates the timing of differentiation (52). In fact, maturation of several cell types in the brain in development may depend on specific windows of TRα1 expression and involve a complicated interplay between TRs, THs, and coregulators (2). Additionally, TRα1 is known to exhibit important roles in later stages of neurodevelopment and its expression persists in adult neurons. Therefore, it is interesting that expression of the TRα1 isoform predominates in both fetal and in adult brain at the same times when free T_4 levels appear to be at biologically active levels (16), suggesting windows in brain development may exist where T_4 may act on TRα1.

DEIODINASE 2-DEFICIENT MICE EXHIBIT NORMAL CNS DEVELOPMENT AND FUNCTION

As noted above, deiodinase 2 expression does not overlap TR receptor expression in the brain. Deiodinase 2 is expressed instead in astrocytes whereas the TRs are expressed in neurons along with deiodinase 3 [(28, 29); **Figure 2**]. The current theory therefore suggests astrocytes are involved with T_4 uptake from capillaries to subsequently generate a source of locally generated T_3. Conversion of T_4 into T_3 via deiodinase 2 in astrocytes has been estimated to

produce as much as 80% of the T_3 bound to the TRs in the brain (18), suggesting astrocyte deiodinase 2 is important for generating local T_3 concentrations. Therefore, many argue that deiodinase 2 likely plays a critical role in developing brain by providing the necessary amount of T_3. If this were in fact the case, one would predict the absence of deiodinase 2 would result in detrimental defects in CNS development similar to that seen in hypothyroidism.

However, the Galton lab produced a deiodinase 2-deficient and a deiodinase 2/deiodinase 1 dual-deficient mouse (KOs) without any evident defects in brain development or function (27, 53). The deiodinase KO mice demonstrated slightly elevated circulating T_4 and TSH levels, and normal thyroid-secretion of T_3 but no tissue-level production of T_3 from T_4 (27). Notably, these mice did not display any signs of hypothyroidism and have no gross physiological or behavioral abnormalities (27). The deiodinase KO was also combined with an MCT8 TH transporter knockout (54, 55); this combination resulted in minor neuronal defects mostly noted by decreased expression of genes in the neural cortex, which are usually positively regulated by T_3, however, most neural development and function was normal. KO mice studies suggest that T_3 transport into the brain and local conversion of T_4 to T_3 in the brain are not essential for normal brain function in mice, and suggest that CNS T_3-defects do not produce syndromes as severe as that seen in the hypothyroid mice (27).

Many suggest that there might be compensation in the deiodinase KO mice through the absorption of more T_3 directly from circulation via the MCT8 transporter in endothelial cells of the BBB, but it should be again noted that the parallel transporters such as OATP1C1 and OATP2 favor T_4 transport (56, 57) and it is unlikely that T_3 can be transported into the brain at rate equivalent to T_4 transport. We suggest that in the absence of available T_3, T_4 can act as an active TH in the brain working on, most likely, TRα1. Interestingly, in the absence of deiodinase 1 and 2, positively regulated TH genes in the cerebral cortex remain unaffected but negatively regulated TH genes appear to be impaired in a way that parallel the hypothyroid mice (27, 58). Perhaps in the absence of deiodinase 2, T_4 can act as an active hormone in brain cells to activate positively regulated TH genes, but not to repress negatively regulated TH genes.

It should be noted that humans with MCT8 mutations display severe neurodevelopmental defects with psychomotor retardation and abnormal serum TH levels (57, 59)). Contrastingly, MCT8 KO mice mimic the human MCT8 mutations in their thyroid phenotype but display no obvious brain developmental defects (57, 59). It is therefore possible that the need for locally produced T_3, and/or the presence of alternative T3-specific transporters, differ in mice and in humans (55).

TR COREGULATORS AND THE BRAIN

T_4 efficiently recruits many coactivators to TRα1, with certain well-established TR coactivators (SRC1 and TRAP220) exhibiting a T_4 response equal or near equal to that induced by T_3 (Amy C. Schroeder and Martin L. Privalsky, unpublished data). SRC1 mRNA is expressed in many tissues during development including the CNS (60). TRAP220 is also expressed in the developing brain and is thought to play a regulatory role in the process of cell proliferation and differentiation, in learning, and in memory formation (61). The widespread expression of TRAP220 in the developing brain appears to parallel TRα1 expression. Therefore, CNS development correlates with a high level of expression of TRα1 together with TRAP220 and/or SRC1 and may provide an opportunity for T_4 to directly regulate gene transcription. CNS cell-specific differences in TR isoform and cofactor levels or function are likely to contribute to differences in T_4 hormone response and may suggest a means by which the T_4 sensitivity of a given CNS cell type can be regulated in response to internal or external signals.

A POSSIBLE DIRECT ROLE FOR T_4 IN BRAIN: ARE THERE CONTEXTS IN THE BRAIN IN WHICH T_4 IS A DIRECT-ACTING TRα1 AGONIST?

Several recent studies have led to the view that T_4 exhibits non-genomic roles that do not require conversion to T_3 (20) but which have not challenged the general view that T_3, not T_4, is the only direct, biologically relevant agonist for nuclear TR function. Our own experiments indicate that TRα1 has the potential to act as a dual sensor of both T_4 and T_3 (Amy C. Schroeder and Martin L. Privalsky, unpublished observations).

Although the effective concentration of T_4 in the brain is difficult to determine, it is plausible that T_4 levels are sufficient to induce activation of TRα1-regulated genes in the brain even in the absence of T_3. We suggest that the normal mix of T_4 and T_3 in the brain may actually confer a mixed T_4/T_3 transcription response mediated primarily by TRα1, together with a more pure T_3 response mediated primarily by TRβ1. Notably, mice in which both deiodinase 1 and 2 have been genetically ablated, and thus lack astrocyte deiodinase conversion of T_4 to T_3, display only very mild defects in their physiological with little to no neurological defects (27). If, as indicated by these knockouts, T_4 is not absolutely required in its traditional role as a pro-hormone, the dominance of T_4 to T_3 in the circulation and transport into the CNS may instead reflect a novel role of T_4 as a direct-acting hormone and this direct role may be helping to ameliorate the effects of the deiodinase knockouts in the CNS.

In conclusion, TH endocrinology in the CNS is tightly regulated at multiple tiers. Negative feedback loops in the hypothalamus and the pituitary control T_3 and T_4 output by the thyroid gland itself. Further, multiple phenomenon functions together to modulate the transport of circulating TH through the BBB, and multiple transporters act together to directly alter TH availability in the CNS itself. Additionally, conversion of intracellular T_4 into T_3 by deiodinase 2, inactivation of both T_3 and T_4 by deiodinase 3, and, the ability of different TR isoforms and different coregulators to respond directly to T_4 versus T_3 further regulate the CNS response to TH. Operating together, we propose these mechanisms serve to maintain proper endocrine homeostasis while permitting the CNS to respond to developmental and physiological needs.

REFERENCES

1. 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. doi:10.1530/eje.0.151U025
2. 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**:1904–16. doi:10.1210/me.2010-0175

3. Galton VA. The roles of the iodothyronine deiodinases in mammalian development. *Thyroid* (2005) **15**:823–34. doi:10.1089/thy.2005.15.823
4. Laurberg P. Thyroid function: thyroid hormones, iodine and the brain-an important concern. *Nat Rev Endocrinol* (2009) **5**:475–6. doi:10.1038/nrendo.2009.155
5. Arrojo EDR, Fonseca TL, Werneck-De-Castro JP, Bianco AC. Role of the type 2 iodothyronine deiodinase (D2) in the control of thyroid hormone signaling. *Biochim Biophys Acta* (2013) **1830**:3956–64. doi:10.1016/j.bbagen.2012.08.019
6. DeLong GR, Stanbury JB, Fierro-Benitez R. Neurological signs in congenital iodine-deficiency disorder (endemic cretinism). *Dev Med Child Neurol* (1985) **27**:317–24. doi:10.1111/j.1469-8749.1985.tb04542.x
7. Thompson CC, Potter GB. Thyroid hormone action in neural development. *Cereb Cortex* (2000) **10**:939–45. doi:10.1093/cercor/10.10.939
8. Quignodon L, Legrand C, Allioli N, Guadano-Ferraz A, Bernal J, Samarut J, et al. Thyroid hormone signaling is highly heterogeneous during pre- and postnatal brain development. *J Mol Endocrinol* (2004) **33**:467–76. doi:10.1677/jme.1.01570
9. Gereben B, Zeold A, Dentice M, Salvatore D, Bianco AC. Activation and inactivation of thyroid hormone by deiodinases: local action with general consequences. *Cell Mol Life Sci* (2008) **65**:570–90. doi:10.1007/s00018-007-7396-0
10. St Germain DL, Galton VA, Hernandez A. Minireview: defining the roles of the iodothyronine deiodinases: current concepts and challenges. *Endocrinology* (2009) **150**:1097–107. doi:10.1210/en.2008-1588
11. Yen PM. Thorough physiological review – physiological and molecular basis of thyroid hormone action. *Physiol Rev* (2001) **81**:1097–142.
12. Sandler B, Webb P, Apriletti JW, Huber BR, Togashi M, Cunha Lima ST, et al. Thyroxine-thyroid hormone receptor interactions. *J Biol Chem* (2004) **279**:55801–8. doi:10.1074/jbc.M410124200
13. Larsen PR, Davies TF, Schlumberger M-J, Hay ID. Thyroid physiology and diagnostic evaluation of patients with thyroid disorders. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, editors. *Williams Textbook of Endocrinology*. Philadelphia: Saunders (2003). p. 331–73.
14. Galton VA. The role of 3,5,3′-triiodothyronine in the physiological action of thyroxine in the premetamorphic tadpole. *Endocrinology* (1989) **124**:2427–33. doi:10.1210/endo-124-5-2427
15. Zimmermann MB. The impact of iodised salt or iodine supplements on iodine status during pregnancy, lactation and infancy. *Public Health Nutr* (2007) **10**:1584–95. doi:10.1017/S1368980007360965
16. de Escobar GM, Ares S, Berbel P, Obregon MJ, Del Rey FE. The changing role of maternal thyroid hormone in fetal brain development. *Semin Perinatol* (2008) **32**:380–6. doi:10.1053/j.semperi.2008.09.002
17. Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, et al. Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. *Thyroid* (2011) **21**:1081–125. doi:10.1089/thy.2011.0087
18. 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**:367–75. doi:10.1210/endo-110-2-367
19. Heuer H. The importance of thyroid hormone transporters for brain development and function. *Best Pract Res Clin Endocrinol Metab* (2007) **21**:265–76. doi:10.1016/j.beem.2007.03.003
20. Davis PJ, Davis FB, Lin HY, Mousa SA, Zhou M, Luidens MK. Translational implications of nongenomic actions of thyroid hormone initiated at its integrin receptor. *Am J Physiol Endocrinol Metab* (2009) **297**:E1238–46. doi:10.1152/ajpendo.00480.2009
21. O'Shea PJ, Williams GR. Insight into the physiological actions of thyroid hormone receptors from genetically modified mice. *J Endocrinol* (2002) **175**:553–70. doi:10.1677/joe.0.1750553
22. Williams RH, Larsen PR. *Williams Textbook of Endocrinology*. Philadelphia, PA: Saunders (2003).
23. Friesema EC, Ganguly S, Abdalla A, Manning Fox JE, Halestrap AP, Visser TJ. Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. *J Biol Chem* (2003) **278**:40128–35. doi:10.1074/jbc.M300909200
24. 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**:1357–69. doi:10.1210/me.2007-0112
25. Bianco AC, Kim BW. Deiodinases: implications of the local control of thyroid hormone action. *J Clin Invest* (2006) **116**:2571–9. doi:10.1172/JCI29812
26. Chan SY, Vasilopoulou E, Kilby MD. The role of the placenta in thyroid hormone delivery to the fetus. *Nat Clin Pract Endocrinol Metab* (2009) **5**:45–54. doi:10.1038/ncpendmet1026
27. Galton VA, Schneider MJ, Clark AS, St Germain DL. Life without thyroxine to 3,5,3′-triiodothyronine conversion: studies in mice devoid of the 5′-deiodinases. *Endocrinology* (2009) **150**:2957–63. doi:10.1210/en.2008-1572
28. 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. doi:10.1007/BF00236998
29. Tu HM, Kim SW, Salvatore D, Bartha T, Legradi G, Larsen PR, et al. Regional distribution of type 2 thyroxine deiodinase messenger ribonucleic acid in rat hypothalamus and pituitary and its regulation by thyroid hormone. *Endocrinology* (1997) **138**:3359–68. doi:10.1210/endo.138.8.5318
30. Guadano-Ferraz A, Escamez 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**:3430–9.
31. Calvo R, Obregon MJ, Ruiz, De Ona 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**:889–99. doi:10.1172/JCI114790
32. 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**:229–36. doi:10.1016/0006-8993(91)90194-Z
33. Chatonnet F, Picou F, Fauquier T, Flamant F. Thyroid hormone action in cerebellum and cerebral cortex development. *J Thyroid Res* (2011) **2011**:145762. doi:10.4061/2011/145762
34. 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**:1094–103. doi:10.1002/glia.20990
35. Lazar MA, Berrodin TJ, Harding HP. Differential DNA binding by monomeric, homodimeric, and potentially heteromeric forms of the thyroid hormone receptor. *Mol Cell Biol* (1991) **11**:5005–15.
36. Naar AM, Boutin JM, Lipkin SM, Yu VC, Holloway JM, Glass CK, et al. The orientation and spacing of core DNA-binding motifs dictate selective transcriptional responses to three nuclear receptors. *Cell* (1991) **65**:1267–79. doi:10.1016/0092-8674(91)90021-P
37. Forman BM, Casanova J, Raaka BM, Ghysdael J, Samuels HH. Half-site spacing and orientation determines whether thyroid hormone and retinoic acid receptors and related factors bind to DNA response elements as monomers, homodimers, or heterodimers. *Mol Endocrinol* (1992) **6**:429–42. doi:10.1210/mend.6.3.1316541
38. Wahlstrom GM, Sjoberg M, Andersson M, Nordstrom K, Vennstrom B. Binding characteristics of the thyroid hormone receptor homo- and heterodimers to consensus AGGTCA repeat motifs. *Mol Endocrinol* (1992) **6**:1013–22. doi:10.1210/me.6.7.1013
39. Kurokawa R, Yu VC, Naar A, Kyakumoto S, Han Z, Silverman S, et al. Differential orientations of the DNA-binding domain and carboxy-terminal dimerization interface regulate binding site selection by nuclear receptor heterodimers. *Genes Dev* (1993) **7**:1423–35. doi:10.1101/gad.7.7b.1423
40. Privalsky ML, Lee S, Hahm JB, Young BM, Fong RN, Chan IH. The p160 coactivator PAS-B motif stabilizes nuclear receptor binding and contributes to isoform-specific regulation by thyroid hormone receptors. *J Biol Chem* (2009) **284**:19554–63. doi:10.1074/jbc.M109.007542
41. Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. *Endocr Rev* (2010) **31**:139–70. doi:10.1210/er.2009-0007
42. Rosen MD, Privalsky ML. Thyroid hormone receptor mutations found in renal clear cell carcinomas alter corepressor release and reveal helix 12 as key determinant of corepressor specificity. *Mol Endocrinol* (2009) **23**:1183–92. doi:10.1210/me.2009-0126
43. Chan IH, Privalsky ML. Isoform-specific transcriptional activity of overlapping target genes that respond to thyroid hormone receptors alpha1 and beta1. *Mol Endocrinol* (2009) **23**:1758–75. doi:10.1210/me.2009-0025

44. Chatonnet F, Guyot R, Benoit G, Flamant F. Genome-wide analysis of thyroid hormone receptors shared and specific functions in neural cells. *Proc Natl Acad Sci U S A* (2013) **110**:E766–75. doi:10.1073/pnas.1210626110
45. Murata Y. Multiple isoforms of thyroid hormone receptor: an analysis of their relative contribution in mediating thyroid hormone action. *Nagoya J Med Sci* (1998) **61**:103–15.
46. Forrest D, Vennstrom B. Functions of thyroid hormone receptors in mice. *Thyroid* (2000) **10**:41–52. doi:10.1089/thy.2000.10.41
47. Zhang J, Lazar MA. The mechanism of action of thyroid hormones. *Annu Rev Physiol* (2000) **62**:439–66. doi:10.1146/annurev.physiol.62.1.439
48. Wondisford FE. Thyroid hormone action: insight from transgenic mouse models. *J Investig Med* (2003) **51**:215–20. doi:10.2310/6650.2003.39191
49. Cheng SY. Isoform-dependent actions of thyroid hormone nuclear receptors: lessons from knockin mutant mice. *Steroids* (2005) **70**:450–4. doi:10.1016/j.steroids.2005.02.003
50. Flamant F, Samarut J. Thyroid hormone receptors: lessons from knockout and knock-in mutant mice. *Trends Endocrinol Metab* (2003) **14**:85–90. doi:10.1016/S1043-2760(02)00043-7
51. Schwartz HL, Strait KA, Ling NC, Oppenheimer JH. Quantitation of rat tissue thyroid hormone binding receptor isoforms by immunoprecipitation of nuclear triiodothyronine binding capacity. *J Biol Chem* (1992) **267**:11794–9.
52. Heuer H, Mason CA. Thyroid hormone induces cerebellar Purkinje cell dendritic development via the thyroid hormone receptor alpha1. *J Neurosci* (2003) **23**:10604–12.
53. Schneider MJ, Fiering SN, Pallud SE, Parlow AF, St Germain DL, Galton VA. Targeted disruption of the type 2 selenodeiodinase gene (DIO2) results in a phenotype of pituitary resistance to T4. *Mol Endocrinol* (2001) **15**:2137–48. doi:10.1210/me.15.12.2137
54. Liao XH, Di Cosmo C, Dumitrescu AM, Hernandez A, Van Sande J, St Germain DL, et al. Distinct roles of deiodinases on the phenotype of MCT8 defect: a comparison of eight different mouse genotypes. *Endocrinology* (2011) **152**:1180–91. doi:10.1210/en.2010-0900
55. Morte B, Ceballos A, Diez D, Grijota-Martinez C, Dumitrescu AM, Di Cosmo C, et al. 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**:2381–7. doi:10.1210/en.2009-0944
56. Roberts LM, Woodford K, Zhou M, Black DS, Haggerty JE, Tate EH, et al. Expression of the thyroid hormone transporters monocarboxylate transporter-8 (SLC16A2) and organic ion transporter-14 (SLCO1C1) at the blood-brain barrier. *Endocrinology* (2008) **149**:6251–61. doi:10.1210/en.2008-0378
57. Ceballos A, Belinchon MM, Sanchez-Mendoza E, Grijota-Martinez C, Dumitrescu AM, Refetoff S, et al. Importance of monocarboxylate transporter 8 for the blood-brain barrier-dependent availability of 3,5,3′-triiodo-L-thyronine. *Endocrinology* (2009) **150**:2491–6. doi:10.1210/en.2008-1616
58. 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**:2919–28. doi:10.1210/en.2011-1905
59. Ferrara AM, Liao XH, Gil-Ibanez P, Marcinkowski T, Bernal J, Weiss RE, et al. Changes in thyroid status during perinatal development of MCT8-deficient male mice. *Endocrinology* (2013) **154**:2533–41. doi:10.1210/en.2012-2031
60. Iannacone EA, Yan AW, Gauger KJ, Dowling AL, Zoeller RT. Thyroid hormone exerts site-specific effects on SRC-1 and NCoR expression selectively in the neonatal rat brain. *Mol Cell Endocrinol* (2002) **186**:49–59. doi:10.1016/S0303-7207(01)00672-4
61. Galeeva A, Treuter E, Tuohimaa P, Pelto-Huikko M. Comparative distribution of the mammalian mediator subunit thyroid hormone receptor-associated protein (TRAP220) mRNA in developing and adult rodent brain. *Eur J Neurosci* (2002) **16**:671–83. doi:10.1046/j.1460-9568.2002.02115.x

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Thyroid hormone and seasonal rhythmicity

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Living organisms show seasonality in a wide array of functions such as reproduction, fattening, hibernation, and migration. At temperate latitudes, changes in photoperiod maintain the alignment of annual rhythms with predictable changes in the environment. The appropriate physiological response to changing photoperiod in mammals requires retinal detection of light and pineal secretion of melatonin, but extraretinal detection of light occurs in birds. A common mechanism across all vertebrates is that these photoperiod-regulated systems alter hypothalamic thyroid hormone (TH) conversion. Here, we review the evidence that a circadian clock within the pars tuberalis of the adenohypophysis links photoperiod decoding to local changes of TH signaling within the medio-basal hypothalamus (MBH) through a conserved thyrotropin/deiodinase axis. We also focus on recent findings which indicate that, beyond the photoperiodic control of its conversion, TH might also be involved in longer-term timing processes of seasonal programs. Finally, we examine the potential implication of kisspeptin and RFRP3, two RF-amide peptides expressed within the MBH, in seasonal rhythmicity.

Keywords: seasonality, reproduction, pars tuberalis, melatonin rhythm, kisspeptins, RF-amide, GnRH neurons

INTRODUCTION

Seasonality is a critical property of most organisms. At temperate latitudes, photoperiod is the main synchronizer of seasonal functions. Photoperiodism defines the use of the annual cycle of day and night length to coordinate functions such as reproduction, fattening, hibernation, and migration with predictable changes in the environment, for example in food availability or climatic conditions. Seasonal changes in physiology and behavior typically are innately timed long-term processes, requiring weeks or months to wax and wane. Therefore, additional to photoperiodic readout mechanisms, living creatures have evolved endogenous long-term timing devices, which allow them to anticipate forthcoming seasonal changes. In the most extreme cases, cycles of about 365 days recur for years in animals kept under constant photoperiods; such so-called circannual rhythms exist in a variety of birds and longer-lived mammals.

Species with relatively short life spans such as voles and hamsters usually do not display circannual rhythms, but their seasonal cycles also comprise an endogenously generated part, which corresponds to the overwintering period and allows timely emergence from the burrow and reproductive recrudescence in early spring. Endogenous long-term timing is commonplace in vertebrates but its mechanistic basis remains mysterious [for reviews, see Ref. (1–6)]. Here we review findings, essentially in birds and mammals, which clarify the mechanisms of photoperiodic readout and provide a rationale for the seasonal control of thyroid hormone (TH) metabolism within the hypothalamus.

PHOTOPERIODISM: MELATONIN AND THE PARS TUBERALIS

The crucial role of melatonin in mammalian photoperiodism has been established in many species including hamsters, ferrets, and sheep (7–9). Within the pineal, melatonin is produced and released during the night and therefore constitutes an internal neurochemical representation of photoperiod. Timed melatonin-infusion experiments established that duration is the key parameter of the melatonin pattern that triggers the photoperiodic response [for review, see Ref. (10)]. In order to map central binding sites, autoradiography with 2-iodo-melatonin was used in a wide range of mammals (11). Surprisingly, across all species the highest density of melatonin-binding sites was found in the *pars tuberalis* (PT), a region of the pituitary stalk apposed to the median eminence. The suprachiasmatic nuclei (SCN) also showed moderate labeling in most species while many brain nuclei showed weak to moderate labeling, with very little species overlap [for reviews, see Ref. (12, 13)]. The presence of melatonin receptors within the SCN was consistent with the effects of melatonin on daily timing in mammals (14). Conversely, since the PT was the only neuroendocrine structure labeled in the highly photoperiodic ferret, a role in seasonality was anticipated (15). However, melatonin-binding sites were also disclosed within the PT of species, which are not overtly photoperiodic such as mouse, rat, and human.

Melatonin-binding studies also led to the recognition that the binding site(s) for melatonin was a classical GPCR, with picomolar affinity for its ligand. In mammals, two high-affinity melatonin receptors (MT1 and MT2) were cloned (16, 17). Subsequent studies showed that MT1 is the predominant subtype, both necessary

and sufficient to mediate the photoperiodic effect of melatonin (18–22). The number of central sites expressing melatonin receptors as revealed by *in situ* hybridization was comparatively more restricted – mostly the PT and the SCN – than that observed with melatonin-binding studies. This may reflect the difference in sensitivity of the techniques and/or the existence of a low-affinity melatonin-binding site. The latter would be physiologically irrelevant, and probably corresponds to quinone reductase 2 rather than a true melatonin receptor (23).

MELATONIN-DEPENDENT TSH RELEASE IN THE PARS TUBERALIS

The PT is the most rostral part of the adenohypophysis. Many reviews detailing the ontogeny, morphology, and immunohistochemical characteristics of the PT are available (24–28). The PT was once considered an "undifferentiated embryological remnant of the hypophysis" whose "only function is to provide mechanical support role for the hypothalamo-hypophyseal portal vessels" [see Ref. (29)]. However, its location and anatomical features pleaded in favor of a specific role: the PT extends along the ventral aspect of the median eminence, surrounds the pituitary stalk in its most caudal part, and is in contact with nerve endings of the median eminence and capillaries of the pituitary primary plexus.

The PT is phylogenetically conserved in tetrapods, but is generally absent in fish (30), and consists of endocrine cells, which exhibit early secretory activity compared to the pars distalis (PD). Three different cell types occur in the PT: (i) follicular cells; (ii) gonadotropes, which constitute ~10% of the endocrine PT cells, have dense-core granules and occur mostly in the caudal PT (known as the *zona tuberalis*); (iii) PT-specific cells, which are virtually agranular thyrotropes and constitute ~90% of endocrine PT cells. The PT gonadotropes appear identical to those in the PD, while shape and ultrastructure of PT-specific thyrotropes differ strikingly from those in the PD (24, 25). These thyrotropes were therefore suspected to be a peculiar pituitary endocrine cell type, possibly producing a novel glycoprotein ["tuberalin," Ref. (31)]. These cells exhibit early secretory activity compared to PD endocrine cells (32). This depends upon the induction of *Tsh*β transcription by a transcription factor consequently called TEF [Thyrotroph Embryonic Factor; Ref. (33)].

Based on ultrastructure and immunohistochemistry, these PT-specific thyrotropes were predicted to be melatonin-responsive, a prediction which has since been validated (34, 35). TSH immunoreactivity within these cells displays dramatic melatonin-dependent photoperiodic changes, with high and low levels under long (LP) and short photoperiod (SP), respectively (36, 37). Finally, the TSH produced by these PT-specific thyrotropes may be identical to that produced by the PD, but the transcriptional control of the *Tsh*β gene in the two populations differs since PT thyrotropes do not express receptors for either TRH or TH (38). Hence, *Tsh*β expression by PT-specific thyrotropes is disconnected from the classical hypothalamic–pituitary–thyroid axis; instead it depends upon melatonin.

However, considering the Harris dogma of a descending flow of information from the hypothalamus to the pituitary, a role for PT-derived TSH was not forthcoming. Rather, it was assumed that, should the PT play a role in seasonality, it would most probably be to release tuberalin(s) in the pituitary portal plexus, which would then target the PD. This might be the case for the seasonal control of the lactotropic axis, even though the mechanism is unclear (39). This aspect will not be considered further here as it has been discussed elsewhere (28, 40–42).

THYROID HORMONE SIGNALING IN SEASONAL CYCLES

AN OVERVIEW

The pioneering work of Benoit on ducks in the 1930s revealed that the thyroid gland is mandatory for seasonal transitions in reproductive states, a finding which applies to a wide range of vertebrates [reviewed by Nicholls et al. (43); Hazlerigg and Loudon (44); Yoshimura (45)]. Thyroidectomy prevents the cessation of breeding in starlings (46), quail (47), and sheep [Ref. (43, 48, 49); for review, see Ref. (50)]. In rams, thyroidectomy during the non-breeding season almost immediately reactivates the gonadotropic axis (51). Therefore, TH appeared to transmit the message of long-day lengths. Microimplants releasing small amount of TH were then surgically placed within the brain of the ewe (52, 53), which revealed that TH acts centrally, and most likely within the medio-basal hypothalamus (MBH), to impact seasonal reproduction. Studies in Siberian hamsters using a similar microimplants approach further showed that other seasonal axes are also controlled by central actions of T3: providing T3 directly within the MBH overrides the SP-induced inactivation of the gonadotropic axis (54) and triggers premature gonadal recrudescence in SP-exposed animals. T3 implants also override SP-induced seasonal inappetence, weight loss, and expression of torpor [Ref. (55); see **Figure 1**]. Similar outcomes are found when T3 is provided by daily subcutaneous injections to SP-exposed hamsters (56). In contrast to these effects on reproduction and energy metabolism, T3 implants do not impact the lactotropic axis, consistent with a distinct mechanism of control (57, 58) while not incompatible with a common melatonin target tissue as discussed later.

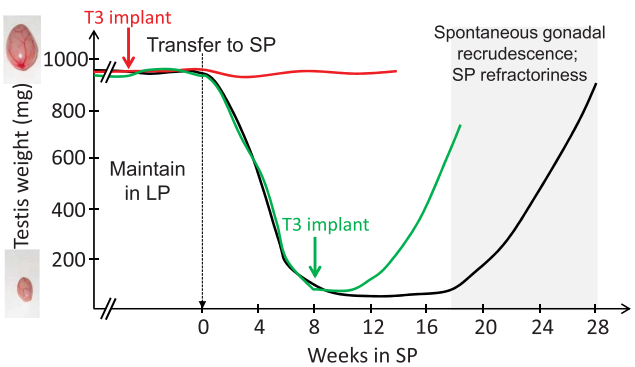

FIGURE 1 | T3 implants prevent SP-induced inactivation of the gonadal axis (red line) and reactivate the gonadal axis in SP-adapted Siberian hamsters [green line; after data from Barrett et al. (54) and Murphy et al. (55)]. Siberian hamsters (black line) kept in LP remain indefinitely sexually active (broken lines) unless they are transferred to SP; gonads then progressively regress (testes depicted here, but data are similar for female reproductive organs). However, prolonged SP exposure leads to a spontaneous recrudescence of the gonads, which reflects SP-refractoriness.

These observations added to the well-documented role of TH in key transitions between life cycles, such as metamorphosis in amphibians and developmental growth and differentiation of the mammalian brain (59, 60). In adults, TH also has key roles in the control of metabolism and thermoregulation, two processes intertwined with the seasonal reproductive cycle. The seasonal program encompasses profound and coordinated changes in behavioral, reproductive, and metabolic states (61). The finding that TH regulates the basal metabolic rate is not new, but the recognition that it reflects a central action within the MBH is very recent (62, 63). Indeed, T3 injection within the MBH suffices to promote food intake and weight gain in rats (64). Interestingly, this effect is mimicked by LP, which triggers weight gain in many species, including sheep (65). Such a process bears critical adaptive value, best exemplified in species that hibernate (e.g., groundhog) or undergo daily torpor (e.g., Siberian hamster), which have evolved a strategy to build up abdominal fat depots during spring/summer to survive the harsh winter season (1, 61). Photoperiodic cues and the metabolic status interact in many seasonal breeders, including sheep (66, 67), goats (68, 69), and horses (70). In all these species, feeding modulates the duration of the breeding season and/or depth of the anestrus. Therefore, TH integrates and coordinates physiological changes, which are integral to the seasonal program.

LOCAL CONTROL OF TH METABOLISM WITHIN THE MBH

Although cold exposure activates thyroid activity, under constant ambient temperature conditions, TH concentrations do not display marked or consistent seasonal fluctuations in the plasma or cerebro-spinal fluid. Rather, fine temporal and local control of TH action is achieved through opposite actions of specific enzymes known as deiodinases (71–73). Deiodinase 2 (DIO2) converts the relatively inactive T4 into the active T3 while deiodinase 3 (DIO3) inactivates T4 by converting it into rT3, and also degrades T3 into T2. Very precise control of T3 concentrations is further achieved through reciprocal control of the expression and activity of these two enzymes by their ligand: a hypothyroid state up-regulates DIO2 and down-regulates DIO3, and vice-versa (72, 74, 75).

The central expression of *Dio2* is restricted to a few structures. The pineal gland is one of them (76), but the strongest expression occurs in astrocytes and tanycytes lining the third ventricle and median eminence (77, 78). These tanycytes also express two major TH transporters, MCT8 and OATP1c1 (79–81), and MCT8 is expressed at higher levels under SP than LP in the Siberian hamster (82, 83). Tanycytes are a heterogeneous and complex population of ependymal cells, which constitute a gateway between the CSF and the MBH and median eminence (84). In a pioneering study, Yoshimura and colleagues (85) showed that both *Dio2* and *Dio3* are expressed within tanycytes of the quail MBH. Crucially, the expression of these two enzymes displays opposite regulation by photoperiod: *Dio2* is highly expressed under LP while *Dio3* is highly expressed under SP. This predicted a local increase of T3 content within the MBH under LP, which was validated by radioimmunoassay (85). The opposite regulation of *Dio2* and *Dio3* by photoperiod has since been described in sparrows and Siberian and Syrian hamsters (86–89). Importantly, the expression of *Dio2* is down-regulated by melatonin, independently of sex steroids (88, 90). Melatonin is also required to trigger *Dio3* expression under SP in Siberian hamster (54). Collectively, these data provided an enzymatic means through which local T3 levels in the MBH could increase under LP.

CLOSING THE LOOP: TSH OUTPUT FROM THE PT GOVERNS T3 REGULATION WITHIN THE MBH

The PT seemed well located to mediate photoperiodic switches in *Dio2–Dio3* usage. To decipher the mechanism of the photoperiodic response, Yoshimura and colleagues (91) set out an ambitious experimental set-up: hypothalamic blocks containing the MBH and PT/median eminence from quails submitted to a long-day transfer, known to activate the gonadotropic axis within 24 h, were used for hybridization on a chicken gene chip. This revealed that the expression of two genes, *Tsh*β and *Eya3*, is rapidly triggered by the transfer from SP to LP. A second wave of transcriptional changes was also observed for a handful of genes including *Dio2* and *Dio3*, which displayed acute and simultaneous induction and repression, respectively. Crucially, expression of the cognate TSH receptor (TSHR) was found in tanycytes, which express the deiodinases, providing the link between TSH output from the PT and T3 regulation within the MBH. The pathway was uncovered using an acute intracerebroventricular injection of TSH to SP-exposed quails, which induced *Dio2* expression and led to gonadal recrudescence.

In a contemporaneous study in sheep, Hanon et al. (92) suggested this mechanism to be ancestral, since their data were similar in many respects: higher *Tsh*β expression within the PT under LP than SP (see **Figure 3A**), expression of the TSHR within tanycytes and PT/median eminence, higher *Dio2* expression under LP than SP (see **Figure 3A**), and TSH-dependent induction of *Dio2* both *in vitro* and *in vivo*. The latter finding was not unexpected, since TSHR signals through a Gs protein, and *Dio2* is a cAMP-responsive gene (93). In contrast, the MT1 receptor couples to a Gi protein and the interplay between TSHR and MT1 signaling within the PT may be part of the photoperiod decoding mechanism, at least in sheep (92, 94, 95). Under LP, the PT therefore functions as an "indirect T3-generator," disconnected from both TRH and T3 feedback (see above).

Since these studies in quail and sheep, a similar TSH/deiodinases/T3 retrograde pathway (from the pituitary back to the hypothalamus, **Figure 2**) has been described not only in other photoperiodic species such as the European hamster (96), the Syrian hamster (97), the Siberian hamster (89), the common vole (98), but also in photoresponsive juvenile Fisher 344 rats (99) and in a melatonin-producing but non-photoperiodic CBA/N

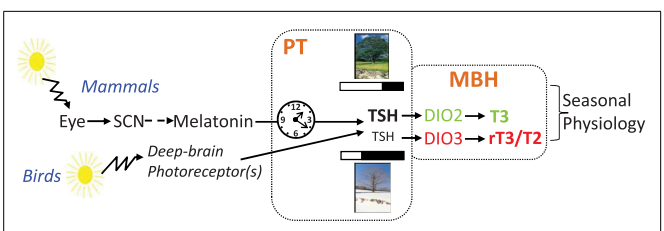

FIGURE 2 | Pathways for photoperiodic entrainment in mammals and birds (see text).

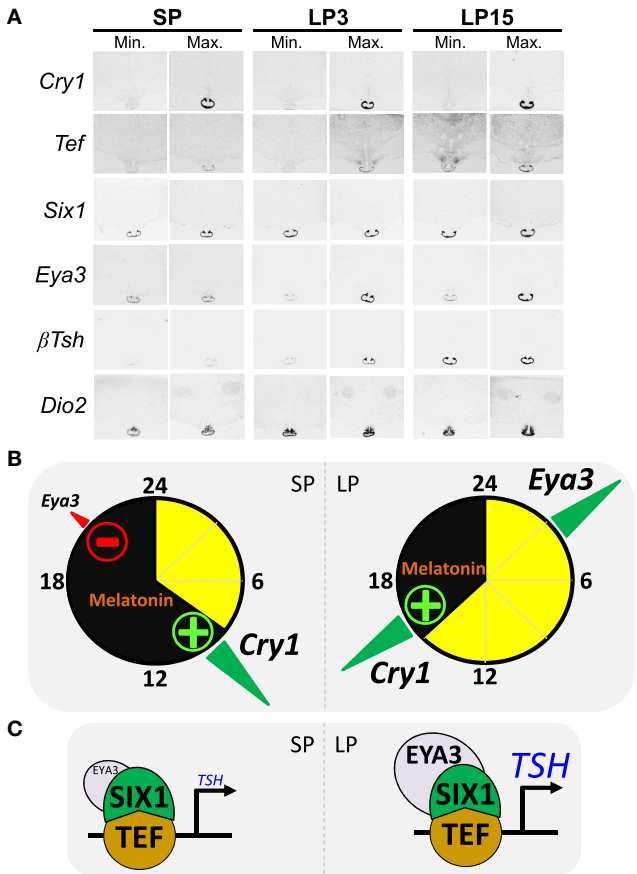

FIGURE 3 | The circadian clock of the *pars tuberalis* links melatonin to the photoperiodic response [after data from Dardente et al. (138) and unpublished data]. **(A)** Images representative of minimal and maximal mRNA levels *in situ* hybridization autoradiograms for *Cry1*, *Tef*, *Six1*, *Eya3*, *Tshβ*, and *Dio2* in sheep kept under SP 8:16 and sheep transferred to LP 16:8 for 3 days (LP3) or 15 days (LP15). **(B)** The internal coincidence model for photoperiodic time-measurement within the PT; SP situation on the left side, LP on the right side, yellow and black indicate day and night. The transcription of *Eya3* is both clock-controlled and inhibited by melatonin, hence the phase-relationship relative to *Cry1* expression (a melatonin-induced circadian gene) is similar irrespective of the photoperiod but *Eya3* transcription increases only under LP as melatonin inhibition is relieved. **(C)** Schematics of the transcriptional control of the *Tshβ* gene by TEF/SIX1/EYA3. Note that EYA3 levels are higher under LP than SP.

mouse strain (22, 100, 101). Photoperiodic variations in *Dio2* expression were however not observed in the non-photoperiodic Wistar rat (88). The use of murine knock-out strains confirmed that the MT1 melatonin receptor (22) and TSHR (100) are mandatory for the LP induction of *Dio2* expression within tanycytes. Whether this pathway is present in all vertebrates remains to be determined (102).

As mentioned before, fish species investigated thus far do not have a distinct PT, but in masu salmon a TSH/DIO2 axis implicating the *saccus vasculosus*, located below the hypothalamus and caudally to the pituitary gland, has been proposed (103). However, the *saccus vasculosus* is absent in several species of fish such as the pike (104), which is nonetheless photoperiodic (105). The other few studies on this matter in fish have yielded varied outcomes (106, 107). Regarding birds, studies in tits (108) and starlings (109) did not lend clear support to the model, but aspects of the experimental set-up prevent any conclusion to be drawn. For example, the studies of starlings were carried out in outdoor aviaries, so effects of fluctuating temperature on the peripheral thyroid axis may have obscured the photoperiodic regulation of DIO2 and DIO3 centrally. Finally, we are not aware of any study on this matter in either reptiles or amphibians.

ENCODING AND DECODING THE PHOTOPERIODIC MESSAGE

UPSTREAM OF THE PT

Birds and mammals possess a similar mechanism to respond to photoperiod, but they perceive the photoperiodic message in different ways. In mammals, light is exclusively perceived by the retina, with a key role for ganglion cells expressing the photopigment melanopsin [for review, see Ref. (110)]. This information is relayed to the circadian clock of the SCN, which governs melatonin production by the pineal gland through a multi-synaptic sympathetic pathway. Melatonin is the mandatory messenger of photoperiod in mammals. In striking contrast, removing the eyes and suppressing melatonin by pinealectomy does not disrupt photoperiodism in birds [for reviews, see Ref. (44, 45, 111–113)]. In birds, light goes through the skull and acts directly upon hypothalamic deep-brain photoreceptors to control seasonal reproduction (**Figure 2**). Several photopigments expressed by different cell types, all located within the MBH and projecting to the PT/median eminence, are plausible candidates: VA-opsin (114), neuropsin [Opn5, Ref. (115, 116)], and melanopsin [Opn4, Ref. (117)]. The neurotransmitter(s) and/or neuropeptide(s) used by these cells, and how they impinge on PT thyrotropes, remain to be elucidated.

WITHIN THE PT: FROM THE CIRCADIAN CLOCK TO THE SEASONAL OUTPUT

Photoperiodic species such as quail (118) and Siberian and Syrian hamsters (119, 120) measure photoperiod length with remarkable accuracy. In these three species, reproduction switches off when the photoperiod is shorter than 12.5 h. The narrow photoperiod range over which physiological changes occur is one of the lines of evidence implicating some sort of daily timing device. The concept that circadian clock(s), clocks with a period of about 24 h, control seasonal timing is indeed not novel (120, 121).

The genetic and molecular bases and organization of circadian clocks have been recently identified (122–124). These clocks are not only located within the SCN, but are present in virtually every tissue and cell where they impact "local" physiology. The PT is no exception as it expresses a full set of clock genes and displays persistent circadian rhythmicity *in vitro* [Ref. (125–127); for review, see Ref. (28)]. The SCN and peripheral clocks share fundamental characteristics: they are cell-autonomous and self-sustained. However, individual cellular clocks within peripheral tissues rapidly become desynchronized and exhibit phase drifting in the absence of regular resetting by cues emanating, directly or indirectly, from the SCN. These cues include inputs from the autonomic nervous system, temperature cycles, and humoral factors such as glucocorticoids and melatonin.

The PT can be defined as a melatonin-dependent circadian oscillator (28, 50). Resetting of the PT clock by melatonin requires acute induction of *Cry1* expression [Ref. (128, 129), see **Figure 3A**]; CRY1 being a key repressor of the circadian clock (130–132). The acute induction of *Cry1* expression involves EGR1-like factors (133) and the transcription factor NPAS4 (134, 135). In sheep, *Cry1* expression remains tightly linked to the onset of melatonin secretion and by implication night onset, irrespective of the duration of the day length [Ref. (136), see **Figure 3B**]. Interestingly, light given during the night induces *Cry1* expression within the quail PT (137), which suggests a phylogenetically conserved role for *Cry1* in the photoperiodic resetting of the PT clock.

How do we connect melatonin resetting of the PT clock with differential photoperiodic output of TSH and seasonal reproduction? The expression of the transcriptional co-activator EYA3 within the ovine PT displays large photoperiodic changes in both phase and amplitude [Ref. (39, 138); see **Figure 3A**]. Interestingly, *Eya3* was the other gene (besides *Tsh*β) immediately induced in the quail PT during the first long-day release experiment (91). We therefore investigated the transcriptional control of *Eya3* and searched for a link between inductions of both genes. The expression of *Eya3* is clock-controlled, through conserved DNA binding motifs within its promoter, and therefore phase-locked to that of the circadian clock [Ref. (138), see **Figure 3B**]. Because of this, expression peaks during the night under SP but during the day under LP. The amplitude of the peak is higher under LP than SP because melatonin suppresses *Eya3* expression, a suppression which can only occur in SP-exposed animals [Ref. (138); see **Figure 3B**]. Finally, *in vitro* data showed that induction of *Tsh*β expression is triggered by the circadian-controlled transcription factor TEF (33), which then recruits the co-activators SIX1 and EYA3. This leads to a marked increase in transcription under LP due to higher levels of EYA3 [Ref. (102, 138), **Figures 3B,C**]. A critical role for SIX1/EYA3, but not TEF, in the photoperiodic control of *Tsh*β transcription in the mouse PT has been proposed (139, 140).

IS T3 OUTPUT SUFFICIENT TO ELICIT THE FULL SPECTRUM OF SEASONAL CHANGES?

The data reviewed so far are consistent with a crucial role for the TSH output of the PT in driving seasonal changes in T3 availability within the MBH. However, swings in TSH/T3 may not be sufficient to elicit all seasonal changes. As mentioned before, since control of the lactotropic axis does not depend on T3 [for review, see Ref. (50)], complementary mechanisms are indeed expected. Neuromedin U (89, 141), histamine, and VGF secretion (82, 142, 143) may mediate seasonal effects on body weight and metabolism since their synthesis and cognate receptors display expression patterns and seasonal changes reminiscent of those seen for TSH/TSHR. However, since TSH infusion in SP-adapted Siberian hamster restores hypothalamic expression of somatostatin and body weight to LP levels (144), Neuromedin U, histamine, or VGF may be dispensable.

Retinoic acid signaling is also likely to be involved as retinoic acid receptors, transporters, and associated binding proteins display prominent photoperiodic regulation in the ependymal cell layer and posterior arcuate nucleus of Siberian hamsters and juvenile Fischer F344 rats (142, 145, 146). Interestingly, the retinoic X receptor (RXR) can heterodimerize with either the TH receptors (THRα/THRβ) or the retinoic acid related receptor (RAR). The target genes and downstream pathways governed by THR and RAR diverge, and therefore the photoperiodic regulation of RXR/RAR may fine-tune the seasonal adaptation of the metabolic status. From a more general standpoint, the notion that tanycytes coordinate a host of seasonal neuroendocrine cycles including reproduction, metabolism, and hibernation is emerging rapidly [Ref. (147, 148); for reviews, see Ref. (61, 149, 150)].

PHOTOPERIODIC TIMING AND THE CIRCANNUAL CLOCK: T3 AS A UNIFYING COMPONENT?

As mentioned earlier, whether species are classified as photoperiodic (e.g., Siberian and Syrian hamsters) or circannual (e.g., sheep), part of the seasonal cycle is generated endogenously. Hamsters and sheep maintained under constant SP do spontaneously revert to the opposite physiological state after several months. This phenomenon, referred to as "SP refractoriness," is typical of an interval timer/hourglass (5, 151). In contrast, sheep but not Siberian or Syrian hamsters, also display refractoriness to LP. Whether this species difference reflects fundamentally divergent underlying mechanisms is questionable. Indeed, Follett and Nicholls (47) proposed years ago that "it may well be that essentially identical physiological mechanisms underlie the photoperiodic responses of a wide range of vertebrates and that very minor modifications of these can cause surprisingly large (though superficial) changes in the overt responses of the animal in terms of reproduction." These authors devised a model, based on differences in threshold sensitivity, which rationalizes the LP refractoriness process (see **Figure 4**). There are indeed similarities between the photoperiodic control of the seasonal program in photoperiodic and circannual species (43, 50, 152). Siberian or Syrian hamsters and sheep might therefore exemplify "variations on a theme" rather than fundamentally different models.

Because TH is involved in many long-term life cycles events, it seems plausible that photoperiod-induced changes in T3 levels may also trigger more profound long-term changes, culminating weeks to months later. In particular, TH-induced plasticity and

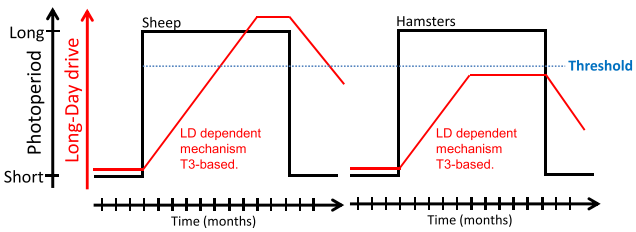

FIGURE 4 | A model for long-day refractoriness [adapted from Figure 2 in Ref. (152)]. In sheep (left panel) and hamsters (right panel), exposure to long days (black line) leads to the development of a mechanism of unknown nature, most likely T3-dependent (red line). In sheep, the long-day drive eventually exceeds a "threshold" (blue dotted line); the animal then becomes refractory to long days and spontaneously reverts to an SP phenotype. In hamsters, the long-day drive never exceeds the threshold and the animal displays the LP phenotype indefinitely; exposure to SP is mandatory to get the SP physiological state.

cell-cycle related events have long time constants, which appear compatible with seasonal cycles (6, 153). Recent data in sheep demonstrate a photoperiodic gating of cell division within the PT and ependymal cells of the 3V and are consistent with this scheme (154–156). Nevertheless, whether photoperiodic gating of cell division depends on TH and/or is involved in seasonal transitions remains to be established.

To address a potential role for TH turn-over beyond the photoperiodic response, we investigated the expression of *Tsh*β and *Dio2*/*Dio3* within the MBH of sheep under distinct physiological states: LP, LP refractory (LPR) obtained after prolonged LP exposure, SP and SP refractory (SPR) obtained after prolonged SP exposure (157). The expressions of *Tsh*β and *Dio2* were diminished in LPR compared to LP animals but remained low in SP and SPR animals. The expression of *Dio3* was high in SP but very low in all other photoperiodic conditions, most notably under SPR; so the expression of *Dio3* under SP is transient (see **Figure 5A**).

Therefore, a diminished TSH output may cause the LPR state, while development of the SPR state would be disconnected from it. This would be consistent with the hourglass properties of the SPR mentioned before. However, changes in *Dio2*/*Dio3* may reflect an indirect effect of photoperiod: within the MBH, the local hyperthyroid state triggered by persistent LP exposure would eventually cross a certain threshold, thereby triggering *Dio3* induction and *Dio2* down-regulation (72, 74, 75). Following this, T3 levels would be cleared by DIO3, ultimately leading to the demise of *Dio3* expression; LP exposure would then somehow be required to induce *Dio2* once more and prime a new cycle. This LP requirement to prime the seasonal sequence may explain why circannual cycles in sheep are most obvious under constant LP (50).

Interestingly, Syrian hamsters in SPR state do not exhibit spontaneous reactivation of *Dio2* expression (88) while Siberian hamsters do (83). Furthermore, Siberian hamsters express *Dio3* upon transfer from LP to SP but its expression is not sustained through time (54, 83, 87), similar to what occurs in sheep (157). Therefore, transient *Dio3* expression under SP appears as a common feature and may explain why *Dio3* expression has not been observed in Syrian hamster (54). Even though the relative variations of *Dio2* and *Dio3* differ between species (e.g., Siberian vs. Syrian hamster) the central tenet remains the same: T3 levels are higher within the MBH under LP compared to SP (158). Furthermore, TH metabolism within the MBH may not only intervene in the photoperiodic response but may also be integral to longer-term timing processes such as circannual rhythms (see **Figure 5B**).

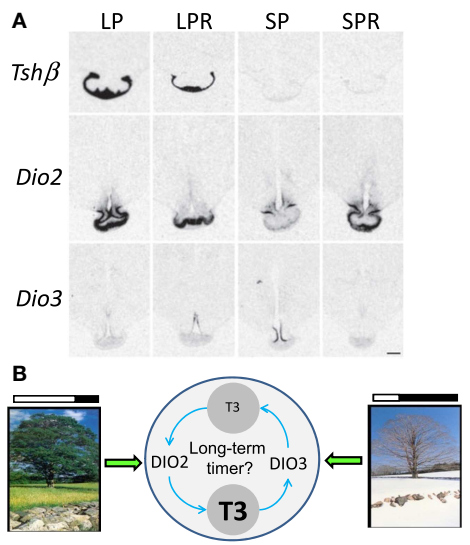

FIGURE 5 | Beyond the long-day response: TH metabolism within the MBH in long-term timing. **(A)** Representative images of *in situ* hybridization autoradiograms for *Tsh*β, *Dio2*, and *Dio3* in sheep under four different endocrine states: LP animals in a spring/summer-like state of reproductive arrest, LP refractory (LPR) animals showing spontaneous reproductive reactivation (late summer/autumn state), SP animals showing autumn/winter-like reproductive activation, and SP refractory (SPR) animals showing spontaneous reproductive arrest [adapted from Saenz de Miera et al. (157)]. **(B)** Schematics depicting (i) the direct effect of LP and SP on DIO2/DIO3 levels, respectively, intertwined with (ii) the possibility that their activity and the resulting TH metabolism constitutes the core of a long-term timing mechanism involved in refractoriness.

CONCLUSION

At this stage several outstanding questions remain: first, since the same TSH/deiodinase/T3 pathway is triggered by LP not only in long-day breeders (e.g., hamsters and quail) but also in short-day breeders (e.g., sheep) and non-photoperiodic species (e.g., mouse), how do we get opposite responses, or no response at all, of the hypothalamic–pituitary–gonadal axis? This is particularly intriguing because the increased intra-hypothalamic availability of TH is uniformly linked to an anabolic state across seasonal species. Second, through which mechanisms do local changes of T3 within the MBH ultimately impinge on gonadotropin-releasing hormone neurons? Pertinent to this second question, the MBH hosts two cell populations expressing RF-amide peptides which have attracted particular attention: neurons of the arcuate nucleus, which express *Kiss1* and neurons of the VMH/DMH, which express the *Rfrp* precursor. The concept that these RF-amide peptides are involved in seasonal breeding has been the topic of several excellent reviews (50, 158–161) and we will therefore only briefly review the most recent and salient findings.

Kisspeptin is a very potent GnRH secretagogue and governs most aspects of reproduction in mammals including sexual differentiation, steroid-dependent gonadotropin release, puberty onset, and the control of fertility by metabolic cues (162, 163). Interestingly, the annual onset of fertility in photoperiodic species had been compared to a reoccurrence of puberty, and common underlying processes were anticipated (2, 164, 165). Kisspeptin therefore appeared a prime candidate for the integration of photoperiodic and metabolic cues across the seasonal program, a prediction which has now received strong support (159–161).

In contrast to kisspeptin, the exact role(s) of peptides derived from the *Rfrp* precursor, RFRP1 and RFRP3, remain(s) unclear (160). RFRP3 may modulate feeding and various stress responses (166, 167). In the context of breeding, RFRP3 inhibits GnRH in sheep [Ref. (168), but see Ref. (169)] but inhibits or activates GnRH in Syrian and Siberian hamsters, depending on the photoperiod (170, 171). The *Rfrp* gene is orthologous to avian *GnIH*, which gives rise to gonadotropin inhibitory hormone (GnIH),

a peptide with well-characterized inhibitory effects upon the gonadotropic axis in birds (172). Interestingly, there is no avian ortholog of the *Kiss1* (or *Kiss2*) gene (173), which implies that the concept of a balance between KISS1 and RFRP3 in governing GnRH secretion in mammals (174) does not apply to birds.

Both *Kiss1* and *Rfrp* expression display marked photoperiodic, melatonin-dependent, changes in mammals (159, 160, 175). Even though melatonin receptors have been localized to several hypothalamic nuclei it seems likely that the photoperiodic control over *Kiss1* and *Rfrp* is indirect [see above, Ref. (50, 145, 161)]. In this context, a role for PT-derived TSH appeared plausible. In a landmark study, Klosen et al. (144) showed that intracerebroventricular delivery of TSH in Siberian and Syrian hamsters induces *Dio2* expression within ependymal cells, restores expression of *Kiss1* and *Rfrp* to their LP levels and, most importantly, triggers reactivation of the gonadal axis. Furthermore, Henson et al. (176) showed that T3 injections to SP-adapted Siberian hamsters reactivated the gonadotropic axis, thereby confirming prior data (see Section "An Overview" and **Figure 1**), but also led to LP-like levels of RF-amide peptides within the MBH.

Therefore, even though a theoretical possibility exists that another TSH-dependent – but T3-independent pathway – leads to seasonal changes of the reproductive axis, the most parsimonious model is one in which T3 action on RF-amide neurons link the photoperiodic production of TSH within the PT to the seasonal control of GnRH secretion.

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REFERENCES

1. Davis DE. Hibernation and circannual rhythms of food consumption in marmots and ground squirrels. *Q Rev Biol* (1976) **51**:477–514. doi:10.1086/409594
2. Lincoln GA, Short RV. Seasonal breeding: nature's contraceptive. *Recent Prog Horm Res* (1980) **36**:1–52.
3. Goldman BD, Darrow JM. The pineal gland and mammalian photoperiodism. *Neuroendocrinology* (1983) **37**:386–96. doi:10.1159/000123579
4. Karsch FJ, Bittman EL, Foster DL, Goodman RL, Legan SJ, Robinson JE. Neuroendocrine basis of seasonal reproduction. *Recent Prog Horm Res* (1984) **40**:185–232.
5. Zucker I. Circannual rhythms. In: Takahashi JS, Turek FW, Moore RY, editors. *Handbook of Behavioural Neurobiology, Circadian Clocks.* (Vol. 12), New York: Kluwer/Plenum (2001). p. 511–28.
6. Lincoln GA, Hazlerigg DG. Mammalian circannual pacemakers. *Soc Reprod Fertil Suppl* (2010) **67**:171–86.
7. Reiter RJ. Evidence for refractoriness of the pituitary-gonadal axis to the pineal gland in golden hamsters and its possible implications in annual reproductive rhythms. *Anat Rec* (1972) **173**:365–71. doi:10.1002/ar.1091730311
8. Carter DS, Herbert J, Stacey PM. Modulation of gonadal activity by timed injections of melatonin in pinealectomized or intact ferrets kept under two photoperiods. *J Endocrinol* (1982) **93**:211–22. doi:10.1677/joe.0.0930211
9. Bittman EL, Dempsey RJ, Karsch FJ. Pineal melatonin secretion drives the reproductive response to daylength in the ewe. *Endocrinology* (1983) **113**:2276–83. doi:10.1210/endo-113-6-2276
10. Bartness TJ, Powers JB, Hastings MH, Bittman EL, Goldman BD. The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses? *J Pineal Res* (1993) **15**:161–90. doi:10.1111/j.1600-079X.1993.tb00903.x
11. Vanecek J, Pavlik A, Illnerova H. Hypothalamic melatonin receptor sites revealed by autoradiography. *Brain Res* (1987) **435**:359–62. doi:10.1016/0006-8993(87)91625-8
12. Morgan PJ, Barrett P, Howell HE, Helliwell R. Melatonin receptors: localization, molecular pharmacology and physiological significance. *Neurochem Int* (1994) **24**:101–46. doi:10.1016/0197-0186(94)90100-7
13. von Gall C, Stehle JH, Weaver DR. Mammalian melatonin receptors: molecular biology and signal transduction. *Cell Tissue Res* (2002) **309**:151–62. doi:10.1007/s00441-002-0581-4
14. Pévet P, Challet E. Melatonin: both master clock output and internal time-giver in the circadian clocks network. *J Physiol Paris* (2011) **105**:170–82. doi:10.1016/j.jphysparis.2011.07.001
15. Weaver DR, Reppert SM. Melatonin receptors are present in the ferret pars tuberalis and pars distalis, but not in brain. *Endocrinology* (1990) **127**:2607–9. doi:10.1210/endo-127-5-2607
16. Reppert SM, Weaver DR, Ebisawa T. Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. *Neuron* (1994) **13**:1177–85. doi:10.1016/0896-6273(94)90055-8
17. Reppert SM, Godson C, Mahle CD, Weaver DR, Slaugenhaupt SA, Gusella JF. Molecular characterization of a second melatonin receptor expressed in human retina and brain: the Mel1b melatonin receptor. *Proc Natl Acad Sci U S A* (1995) **92**:8734–8. doi:10.1073/pnas.92.19.8734
18. Weaver DR, Liu C, Reppert SM. Nature's knockout: the Mel1b receptor is not necessary for reproductive and circadian responses to melatonin in Siberian hamsters. *Mol Endocrinol* (1996) **10**:1478–87. doi:10.1210/me.10.11.1478
19. Liu C, Weaver DR, Jin X, Shearman LP, Pieschl RL, Gribkoff VK, et al. Molecular dissection of two distinct actions of melatonin on the suprachiasmatic circadian clock. *Neuron* (1997) **19**:91–102. doi:10.1016/S0896-6273(00)80350-5
20. Jin X, von Gall C, Pieschl RL, Gribkoff VK, Stehle JH, Reppert SM, et al. Targeted disruption of the mouse Mel(1b) melatonin receptor. *Mol Cell Biol* (2003) **23**:1054–60. doi:10.1128/MCB.23.3.1054-1060.2003
21. Cogé F, Guenin SP, Fery I, Migaud M, Devavry S, Slugocki C, et al. The end of a myth: cloning and characterization of the ovine melatonin MT(2) receptor. *Br J Pharmacol* (2009) **158**:1248–62. doi:10.1111/j.1476-5381.2009.00453.x
22. Yasuo S, Yoshimura T, Ebihara S, Korf HW. Melatonin transmits photoperiodic signals through the MT1 melatonin receptor. *J Neurosci* (2009) **29**:2885–9. doi:10.1523/JNEUROSCI.0145-09.2009
23. Nosjean O, Ferro M, Coge F, Beauverger P, Henlin JM, Lefoulon F, et al. Identification of the melatonin-binding site MT3 as the quinone reductase 2. *J Biol Chem* (2000) **275**:31311–7. doi:10.1074/jbc.M005141200
24. Wittkowski WH, Schulze-Bonhage AH, Bockers TM. The pars tuberalis of the hypophysis: a modulator of the pars distalis? *Acta Endocrinol (Copenh)* (1992) **126**:285–90.
25. Wittkowski W, Bockmann J, Kreutz MR, Bockers TM. Cell and molecular biology of the pars tuberalis of the pituitary. *Int Rev Cytol* (1999) **185**:157–94. doi:10.1016/S0074-7696(08)60151-5
26. Morgan PJ. The pars tuberalis: the missing link in the photoperiodic regulation of prolactin secretion? *J Neuroendocrinol* (2000) **12**:287–95. doi:10.1046/j.1365-2826.2000.00459.x
27. Hazlerigg DG. What is the role of melatonin within the anterior pituitary? *J Endocrinol* (2001) **170**:493–501. doi:10.1677/joe.0.1700493
28. Dardente H. Does a melatonin-dependent circadian oscillator in the pars tuberalis drive prolactin seasonal rhythmicity? *J Neuroendocrinol* (2007) **19**:657–66. doi:10.1111/j.1365-2826.2007.01564.x
29. Gross DS. The mammalian hypophyseal pars tuberalis: a comparative immunocytochemical study. *Gen Comp Endocrinol* (1984) **56**:283–98. doi:10.1016/0016-6480(84)90043-1
30. Fitzgerald KT. The structure and function of the pars tuberalis of the vertebrate adenohypophysis. *Gen Comp Endocrinol* (1979) **37**:383–99. doi:10.1016/0016-6480(79)90012-1
31. Stoeckel ME, Hindelang C, Klein MJ, Poissonnier M, Felix JM. Expression of the alpha-subunit of glycoprotein hormones in the pars tuberalis-specific glandular cells in rat, mouse and guinea-pig. *Cell Tissue Res* (1994) **278**:617–24. doi:10.1007/s004410050253
32. Stoeckel ME, Hindelang-Gertner C, Porte A. Embryonic development and secretory differentiation in the pars tuberalis of the mouse hypophysis. *Cell Tissue Res* (1979) **198**:465–76. doi:10.1007/BF00234191
33. Drolet DW, Scully KM, Simmons DM, Wegner M, Chu KT, Swanson LW, et al. TEF, a transcription factor expressed specifically in the anterior pituitary during

embryogenesis, defines a new class of leucine zipper proteins. *Genes Dev* (1991) **5**:1739–53. doi:10.1101/gad.5.10.1739
34. Klosen P, Bienvenu C, Demarteau O, Dardente H, Guerrero H, Pevet P, et al. The mt1 melatonin receptor and RORbeta receptor are co-localized in specific TSH-immunoreactive cells in the pars tuberalis of the rat pituitary. *J Histochem Cytochem* (2002) **50**:1647–57. doi:10.1177/002215540205001209
35. Dardente H, Klosen P, Pevet P, Masson-Pevet M. MT1 melatonin receptor mRNA expressing cells in the pars tuberalis of the European hamster: effect of photoperiod. *J Neuroendocrinol* (2003) **15**:778–86. doi:10.1046/j.1365-2826.2003.01060.x
36. Wittkowski W, Hewing M, Hoffmann K, Bergmann M, Fechner J. Influence of photoperiod on the ultrastructure of the hypophyseal pars tuberalis of the Djungarian hamster, *Phodopus sungorus. Cell Tissue Res* (1984) **238**:213–6. doi:10.1007/BF00215166
37. Wittkowski W, Bergmann M, Hoffmann K, Pera F. Photoperiod-dependent changes in TSH-like immunoreactivity of cells in the hypophyseal pars tuberalis of the Djungarian hamster, *Phodopus sungorus. Cell Tissue Res* (1988) **251**:183–7. doi:10.1007/BF00215463
38. Bockmann J, Bockers TM, Winter C, Wittkowski W, Winterhoff H, Deufel T, et al. Thyrotropin expression in hypophyseal pars tuberalis-specific cells is 3,5,3'-triiodothyronine, thyrotropin-releasing hormone, and pit-1 independent. *Endocrinology* (1997) **138**:1019–28. doi:10.1210/en.138.3.1019
39. Dupré SM, Miedzinska K, Duval CV, Yu L, Goodman RL, Lincoln GA, et al. Identification of Eya3 and TAC1 as long-day signals in the sheep pituitary. *Curr Biol* (2010) **20**:829–35. doi:10.1016/j.cub.2010.02.066
40. Lincoln GA, Clarke IJ, Hut RA, Hazlerigg DG. Characterizing a mammalian circannual pacemaker. *Science* (2006) **314**:1941–4. doi:10.1126/science.1132009
41. Johnston JD. Photoperiodic regulation of prolactin secretion: changes in intra-pituitary signalling and lactotroph heterogeneity. *J Endocrinol* (2004) **180**:351–6. doi:10.1677/joe.0.1800351
42. Dupré SM. Encoding and decoding photoperiod in the mammalian pars tuberalis. *Neuroendocrinology* (2011) **94**:101–12. doi:10.1159/000328971
43. Nicholls TJ, Follett BK, Goldsmith AR, Pearson H. Possible homologies between photorefractoriness in sheep and birds: the effect of thyroidectomy on the length of the ewe's breeding season. *Reprod Nutr Dev* (1988) **28**:375–85. doi:10.1051/rnd:19880304
44. Hazlerigg D, Loudon A. New insights into ancient seasonal life timers. *Curr Biol* (2008) **18**:R795–804. doi:10.1016/j.cub.2008.07.040
45. Yoshimura T. Neuroendocrine mechanism of seasonal reproduction in birds and mammals. *Anim Sci J* (2010) **81**:403–10. doi:10.1111/j.1740-0929.2010.00777.x
46. Wieselthier AS, Van Tienhoven A. The effect of thyroidectomy on testicular size and on the photorefractory period in the starling (*Sturnus vulgaris* L.). *J Exp Zool* (1972) **179**:331–8. doi:10.1002/jez.1401790306
47. Follett BK, Nicholls TJ. Influences of thyroidectomy and thyroxine replacement on photoperiodically controlled reproduction in quail. *J Endocrinol* (1985) **107**:211–21. doi:10.1677/joe.0.1070211
48. Webster JR, Moenter SM, Barrell GK, Lehman MN, Karsch FJ. Role of the thyroid gland in seasonal reproduction. III. Thyroidectomy blocks seasonal suppression of gonadotropin-releasing hormone secretion in sheep. *Endocrinology* (1991) **129**:1635–43. doi:10.1210/endo-129-3-1635
49. Moenter SM, Woodfill CJ, Karsch FJ. Role of the thyroid gland in seasonal reproduction: thyroidectomy blocks seasonal suppression of reproductive neuroendocrine activity in ewes. *Endocrinology* (1991) **128**:1337–44. doi:10.1210/endo-128-3-1337
50. Dardente H. Melatonin-dependent timing of seasonal reproduction by the pars tuberalis: pivotal roles for long daylengths and thyroid hormones. *J Neuroendocrinol* (2012) **24**:249–66. doi:10.1111/j.1365-2826.2011.02250.x
51. Parkinson TJ, Follett BK. Effect of thyroidectomy upon seasonality in rams. *J Reprod Fertil* (1994) **101**:51–8. doi:10.1530/jrf.0.1010051
52. Viguié C, Battaglia DF, Krasa HB, Thrun LA, Karsch FJ. Thyroid hormones act primarily within the brain to promote the seasonal inhibition of luteinizing hormone secretion in the ewe. *Endocrinology* (1999) **140**:1111–7. doi:10.1210/en.140.3.1111
53. Anderson GM, Hardy SL, Valente M, Billings HJ, Connors JM, Goodman RL. Evidence that thyroid hormones act in the ventromedial preoptic area and the premammillary region of the brain to allow the termination of the breeding season in the ewe. *Endocrinology* (2003) **144**:2892–901. doi:10.1210/en.2003-0322
54. Barrett P, Ebling FJ, Schuhler S, Wilson D, Ross AW, Warner A, et al. Hypothalamic thyroid hormone catabolism acts as a gatekeeper for the seasonal control of body weight and reproduction. *Endocrinology* (2007) **148**:3608–17. doi:10.1210/en.2007-0316
55. Murphy M, Jethwa PH, Warner A, Barrett P, Nilaweera KN, Brameld JM, et al. Effects of manipulating hypothalamic triiodothyronine concentrations on seasonal body weight and torpor cycles in Siberian hamsters. *Endocrinology* (2012) **153**:101–12. doi:10.1210/en.2011-1249
56. Freeman DA, Teubner BJ, Smith CD, Prendergast BJ. Exogenous T3 mimics long day lengths in Siberian hamsters. *Am J Physiol Regul Integr Comp Physiol* (2007) **292**:R2368–72. doi:10.1152/ajpregu.00713.2006
57. Duncan MJ, Goldman BD, Di Pinto MN, Stetson MH. Testicular function and pelage color have different critical daylengths in the Djungarian hamster, *Phodopus sungorus sungorus. Endocrinology* (1985) **116**:424–30. doi:10.1210/endo-116-1-424
58. Maywood ES, Hastings MH. Lesions of the iodomelatonin-binding sites of the mediobasal hypothalamus spare the lactotropic, but block the gonadotropic response of male Syrian hamsters to short photoperiod and to melatonin. *Endocrinology* (1995) **136**:144–53. doi:10.1210/en.136.1.144
59. Galton VA. The roles of the iodothyronine deiodinases in mammalian development. *Thyroid* (2005) **15**:823–34. doi:10.1089/thy.2005.15.823
60. Williams GR. Neurodevelopmental and neurophysiological actions of thyroid hormone. *J Neuroendocrinol* (2008) **20**:784–94. doi:10.1111/j.1365-2826.2008.01733.x
61. Ebling FJ, Barrett P. The regulation of seasonal changes in food intake and body weight. *J Neuroendocrinol* (2008) **20**:827–33. doi:10.1111/j.1365-2826.2008.01721.x
62. Lopez M, Varela L, Vazquez MJ, Rodriguez-Cuenca S, Gonzalez CR, Velagapudi VR, et al. Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. *Nat Med* (2010) **16**:1001–8. doi:10.1038/nm.2207
63. Lopez M, Alvarez CV, Nogueiras R, Dieguez C. Energy balance regulation by thyroid hormones at central level. *Trends Mol Med* (2013) **19**:418–27. doi:10.1016/j.molmed.2013.04.004
64. Kong WM, Martin NM, Smith KL, Gardiner JV, Connoley IP, Stephens DA, et al. Triiodothyronine stimulates food intake via the hypothalamic ventromedial nucleus independent of changes in energy expenditure. *Endocrinology* (2004) **145**:5252–8. doi:10.1210/en.2004-0545
65. Lincoln GA, Rhind SM, Pompolo S, Clarke IJ. Hypothalamic control of photoperiod-induced cycles in food intake, body weight, and metabolic hormones in rams. *Am J Physiol Regul Integr Comp Physiol* (2001) **281**:R76–90.
66. Hulet CV, Shupe WL, Ross T, Richards W. Effects of nutritional environment and ram effect on breeding season in range sheep. *Theriogenology* (1986) **25**:317–23. doi:10.1016/0093-691X(86)90067-1
67. Menassol JB, Collet A, Chesneau D, Malpaux B, Scaramuzzi RJ. The interaction between photoperiod and nutrition and its effects on seasonal rhythms of reproduction in the ewe. *Biol Reprod* (2012) **86**:52. doi:10.1095/biolreprod.111.092817
68. Walkden-Brown SW, Restall BJ, Norton BW, Scaramuzzi RJ, Martin GB. Effect of nutrition on seasonal patterns of LH, FSH and testosterone concentration, testicular mass, sebaceous gland volume and odour in Australian cashmere goats. *J Reprod Fertil* (1994) **102**:351–60.
69. Zarazaga LA, Celi I, Guzman JL, Malpaux B. The role of nutrition in the regulation of luteinizing hormone secretion by the opioidergic, dopaminergic, and serotonergic systems in female Mediterranean goats. *Biol Reprod* (2011) **84**:447–54. doi:10.1095/biolreprod.110.086520
70. Salazar-Ortiz J, Camous S, Briant C, Lardic L, Chesneau D, Guillaume D. Effects of nutritional cues on the duration of the winter anovulatory phase and on associated hormone levels in adult female Welsh pony horses (*Equus caballus*). *Reprod Biol Endocrinol* (2011) **9**:130. doi:10.1186/1477-7827-9-130
71. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr Rev* (2002) **23**:38–89. doi:10.1210/er.23.1.38
72. Lechan RM, Fekete C. Role of thyroid hormone deiodination in the hypothalamus. *Thyroid* (2005) **15**:883–97. doi:10.1089/thy.2005.15.883

73. St Germain DL, Galton VA, Hernandez A. Minireview: defining the roles of the iodothyronine deiodinases: current concepts and challenges. *Endocrinology* (2009) **150**:1097–107. doi:10.1210/en.2008-1588
74. 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**:784–90. doi:10.1210/en.140.2.784
75. Bianco AC, Larsen PR. Cellular and structural biology of the deiodinases. *Thyroid* (2005) **15**:777–86. doi:10.1089/thy.2005.15.777
76. Kalsbeek A, Buijs RM, van Schaik R, Kaptein E, Visser TJ, Doulabi BZ, et al. Daily variations in type II iodothyronine deiodinase activity in the rat brain as controlled by the biological clock. *Endocrinology* (2005) **146**:1418–27. doi:10.1210/en.2004-0763
77. Tu HM, Kim SW, Salvatore D, Bartha T, Legradi G, Larsen PR, et al. Regional distribution of type 2 thyroxine deiodinase messenger ribonucleic acid in rat hypothalamus and pituitary and its regulation by thyroid hormone. *Endocrinology* (1997) **138**:3359–68. doi:10.1210/endo.138.8.5318
78. Guadano-Ferraz A, Obregon MJ, St Germain DL, Bernal J. The type 2 iodothyronine deiodinase is expressed primarily in glial cells in the neonatal rat brain. *Proc Natl Acad Sci U S A* (1997) **94**:10391–6. doi:10.1073/pnas.94.19.10391
79. Nakao N, Takagi T, Iigo M, Tsukamoto T, Yasuo S, Masuda T, et al. Possible involvement of organic anion transporting polypeptide 1c1 in the photoperiodic response of gonads in birds. *Endocrinology* (2006) **147**:1067–73. doi:10.1210/en.2005-1090
80. Ceballos A, Belinchon MM, Sanchez-Mendoza E, Grijota-Martinez C, Dumitrescu AM, Refetoff S, et al. Importance of monocarboxylate transporter 8 for the blood-brain barrier-dependent availability of 3,5,3'-triiodo-L-thyronine. *Endocrinology* (2009) **150**:2491–6. doi:10.1210/en.2008-1616
81. Visser WE, Friesema EC, Visser TJ. Minireview: thyroid hormone transporters: the knowns and the unknowns. *Mol Endocrinol* (2011) **25**:1–14. doi:10.1210/me.2010-0095
82. Herwig A, Wilson D, Logie TJ, Boelen A, Morgan PJ, Mercer JG, et al. Photoperiod and acute energy deficits interact on components of the thyroid hormone system in hypothalamic tanycytes of the Siberian hamster. *Am J Physiol Regul Integr Comp Physiol* (2009) **296**:R1307–15. doi:10.1152/ajpregu.90755.2008
83. Herwig A, de Vries EM, Bolborea M, Wilson D, Mercer JG, Ebling FJ, et al. Hypothalamic ventricular ependymal thyroid hormone deiodinases are an important element of circannual timing in the Siberian hamster (*Phodopus sungorus*). *PLoS One* (2013) **8**:e62003. doi:10.1371/journal.pone.0062003
84. Guerra M, Blazquez JL, Peruzzo B, Pelaez B, Rodriguez S, Toranzo D, et al. Cell organization of the rat pars tuberalis. Evidence for open communication between pars tuberalis cells, cerebrospinal fluid and tanycytes. *Cell Tissue Res* (2010) **339**:359–81. doi:10.1007/s00441-009-0885-8
85. Yoshimura T, Yasuo S, Watanabe M, Iigo M, Yamamura T, Hirunagi K, et al. Light-induced hormone conversion of T4 to T3 regulates photoperiodic response of gonads in birds. *Nature* (2003) **426**:178–81. doi:10.1038/nature02117
86. Watanabe M, Yasuo S, Watanabe T, Yamamura T, Nakao N, Ebihara S, et al. Photoperiodic regulation of type 2 deiodinase gene in Djungarian hamster: possible homologies between avian and mammalian photoperiodic regulation of reproduction. *Endocrinology* (2004) **145**:1546–9. doi:10.1210/en.2003-1593
87. Watanabe T, Yamamura T, Watanabe M, Yasuo S, Nakao N, Dawson A, et al. Hypothalamic expression of thyroid hormone-activating and -inactivating enzyme genes in relation to photorefractoriness in birds and mammals. *Am J Physiol Regul Integr Comp Physiol* (2007) **292**:R568–72. doi:10.1152/ajpregu.00521.2006
88. Revel FG, Saboureau M, Pevet P, Mikkelsen JD, Simonneaux V. Melatonin regulates type 2 deiodinase gene expression in the Syrian hamster. *Endocrinology* (2006) **147**:4680–7. doi:10.1210/en.2006-0606
89. Helfer G, Ross AW, Morgan PJ. Neuromedin U partly mimics thyroid stimulating hormone and triggers Wnt/beta-catenin signalling in the photoperiodic response of F344 rats. *J Neuroendocrinol* (2013) **25**:1264–72. doi:10.1111/jne.12116
90. Yasuo S, Yoshimura T, Ebihara S, Korf HW. Temporal dynamics of type 2 deiodinase expression after melatonin injections in Syrian hamsters. *Endocrinology* (2007) **148**:4385–92. doi:10.1210/en.2007-0497
91. Nakao N, Ono H, Yamamura T, Anraku T, Takagi T, Higashi K, et al. Thyrotrophin in the pars tuberalis triggers photoperiodic response. *Nature* (2008) **452**:317–22. doi:10.1038/nature06738
92. Hanon EA, Lincoln GA, Fustin JM, Dardente H, Masson-Pevet M, Morgan PJ, et al. Ancestral TSH mechanism signals summer in a photoperiodic mammal. *Curr Biol* (2008) **18**:1147–52. doi:10.1016/j.cub.2008.06.076
93. Gereben B, Salvatore D. Pretranslational regulation of type 2 deiodinase. *Thyroid* (2005) **15**:855–64. doi:10.1089/thy.2005.15.855
94. Dupré SM, Dardente H, Birnie MJ, Loudon AS, Lincoln GA, Hazlerigg DG. Evidence for RGS4 modulation of melatonin and thyrotrophin signalling pathways in the pars tuberalis. *J Neuroendocrinol* (2011) **23**:725–32. doi:10.1111/j.1365-2826.2011.02168.x
95. Ebenhoeh O, Hazlerigg D. Modelling a molecular calendar: the seasonal photoperiodic response in mammals. *Chaos Solitons Fractals* (2013) **50**:39–47. doi:10.1016/j.chaos.2012.11.007
96. Hanon EA, Routledge K, Dardente H, Masson-Pevet M, Morgan PJ, Hazlerigg DG. Effect of photoperiod on the thyroid-stimulating hormone neuroendocrine system in the European hamster (*Cricetus cricetus*). *J Neuroendocrinol* (2010) **22**:51–5. doi:10.1111/j.1365-2826.2009.01937.x
97. Yasuo S, Yoshimura T, Ebihara S, Korf HW. Photoperiodic control of TSH-beta expression in the mammalian pars tuberalis has different impacts on the induction and suppression of the hypothalamo-hypophyseal gonadal axis. *J Neuroendocrinol* (2010) **22**:43–50. doi:10.1111/j.1365-2826.2009.01936.x
98. Krol E, Douglas A, Dardente H, Birnie MJ, Vinne V, Eijer WG, et al. Strong pituitary and hypothalamic responses to photoperiod but not to 6-methoxy-2-benzoxazolinone in female common voles (*Microtus arvalis*). *Gen Comp Endocrinol* (2012) **179**:289–95. doi:10.1016/j.ygcen.2012.09.004
99. Ross AW, Helfer G, Russell L, Darras VM, Morgan PJ. Thyroid hormone signalling genes are regulated by photoperiod in the hypothalamus of F344 rats. *PLoS One* (2011) **6**:e21351. doi:10.1371/journal.pone.0021351
100. Ono H, Hoshino Y, Yasuo S, Watanabe M, Nakane Y, Murai A, et al. Involvement of thyrotropin in photoperiodic signal transduction in mice. *Proc Natl Acad Sci U S A* (2008) **105**:18238–42. doi:10.1073/pnas.0808952105
101. Unfried C, Ansari N, Yasuo S, Korf HW, von Gall C. Impact of melatonin and molecular clockwork components on the expression of thyrotropin beta-chain (Tshb) and the TSH receptor in the mouse pars tuberalis. *Endocrinology* (2009) **150**:4653–62. doi:10.1210/en.2009-0609
102. Hazlerigg D. The evolutionary physiology of photoperiodism in vertebrates. *Prog Brain Res* (2012) **199**:413–22. doi:10.1016/B978-0-444-59427-3.00023-X
103. Nakane Y, Ikegami K, Iigo M, Ono H, Takeda K, Takahashi D, et al. The saccus vasculosus of fish is a sensor of seasonal changes in day length. *Nat Commun* (2013) **4**:2108. doi:10.1038/ncomms3108
104. Necrasov O, Adascalitei E. Contribution à l'étude du sac vasculaire des poissons osseux. *Trav Mus Nat His Nat Gr Antipa* (1968) **8**:465–83.
105. Frost WE, Kipling C. A study of reproduction, early life, weight-length relationship and growth of pike, *Esox lucius* L., in Windermere. *J Anim Ecol* (1967) **36**:651–93. doi:10.2307/2820
106. Kitano J, Lema SC, Luckenbach JA, Mori S, Kawagishi Y, Kusakabe M, et al. Adaptive divergence in the thyroid hormone signaling pathway in the stickleback radiation. *Curr Biol* (2010) **20**:2124–30. doi:10.1016/j.cub.2010.10.050
107. O'Brien CS, Bourdo R, Bradshaw WE, Holzapfel CM, Cresko WA. Conservation of the photoperiodic neuroendocrine axis among vertebrates: evidence from the teleost fish, *Gasterosteus aculeatus*. *Gen Comp Endocrinol* (2012) **178**:19–27. doi:10.1016/j.ygcen.2012.03.010
108. Perfito N, Jeong SY, Silverin B, Calisi RM, Bentley GE, Hau M. Anticipating spring: wild populations of great tits (*Parus major*) differ in expression of key genes for photoperiodic time measurement. *PLoS One* (2012) **7**:e34997. doi:10.1371/journal.pone.0034997
109. Bentley GE, Tucker S, Chou H, Hau M, Perfito N. Testicular growth and regression are not correlated with Dio2 expression in a wild male songbird, *Sturnus vulgaris*, exposed to natural changes in photoperiod. *Endocrinology* (2013) **154**:1813–9. doi:10.1210/en.2013-1093
110. Lucas RJ. Mammalian inner retinal photoreception. *Curr Biol* (2013) **23**:R125–33. doi:10.1016/j.cub.2012.12.029
111. Wyse C, Hazlerigg D. Seasonal biology: avian photoreception goes deep. *Curr Biol* (2009) **19**:R685–7. doi:10.1016/j.cub.2009.07.036

112. Ikegami K, Yoshimura T. Seasonal time measurement during reproduction. *J Reprod Dev* (2013) **59**:327–33. doi:10.1262/jrd.2013-035
113. Kosonsiriluk S, Mauro LJ, Chaiworakul V, Chaiseha Y, El Halawani ME. Photoreceptive oscillators within neurons of the premammillary nucleus (PMM) and seasonal reproduction in temperate zone birds. *Gen Comp Endocrinol* (2013) **190**:149–55. doi:10.1016/j.ygcen.2013.02.015
114. Halford S, Pires SS, Turton M, Zheng L, Gonzalez-Menendez I, Davies WL, et al. VA opsin-based photoreceptors in the hypothalamus of birds. *Curr Biol* (2009) **19**:1396–402. doi:10.1016/j.cub.2009.06.066
115. Nakane Y, Ikegami K, Ono H, Yamamoto N, Yoshida S, Hirunagi K, et al. A mammalian neural tissue opsin (opsin 5) is a deep brain photoreceptor in birds. *Proc Natl Acad Sci U S A* (2010) **107**:15264–8. doi:10.1073/pnas.1006393107
116. Yamashita T, Ohuchi H, Tomonari S, Ikeda K, Sakai K, Shichida Y. Opn5 is a UV-sensitive bistable pigment that couples with Gi subtype of G protein. *Proc Natl Acad Sci U S A* (2010) **107**:22084–9. doi:10.1073/pnas.1012498107
117. Kang SW, Leclerc B, Kosonsiriluk S, Mauro LJ, Iwasawa A, El Halawani ME. Melanopsin expression in dopamine-melatonin neurons of the premammillary nucleus of the hypothalamus and seasonal reproduction in birds. *Neuroscience* (2010) **170**:200–13. doi:10.1016/j.neuroscience.2010.06.082
118. Follett BK, Maung SL. Rate of testicular maturation, in relation to gonadotropin and testosterone levels, in quail exposed to various artificial photoperiods and to natural daylengths. *J Endocrinol* (1978) **78**:267–80. doi:10.1677/joe.0.0780267
119. Hoffmann K. The critical photoperiod in the Djungarian hamster *Phodopus sungorus*. In: Aschoff J, Daan S, Groos G, editors. *Vertebrate Circadian Systems*. Heidelberg: Springer-Verlag (1982). p. 297–304.
120. Elliott JA. Circadian rhythms and photoperiodic time measurement in mammals. *Fed Proc* (1976) **35**:2339–46.
121. Pittendrigh CS. Circadian surfaces and the diversity of possible roles of circadian organization in photoperiodic induction. *Proc Natl Acad Sci U S A* (1972) **69**:2734–7. doi:10.1073/pnas.69.9.2734
122. Dardente H, Cermakian N. Molecular circadian rhythms in central and peripheral clocks in mammals. *Chronobiol Int* (2007) **24**:195–213. doi:10.1080/07420520701283693
123. Dibner C, Schibler U, Albrecht U. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. *Annu Rev Physiol* (2010) **72**:517–49. doi:10.1146/annurev-physiol-021909-135821
124. Mohawk JA, Green CB, Takahashi JS. Central and peripheral circadian clocks in mammals. *Annu Rev Neurosci* (2012) **35**:445–62. doi:10.1146/annurev-neuro-060909-153128
125. Messager S, Ross AW, Barrett P, Morgan PJ. Decoding photoperiodic time through Per1 and ICER gene amplitude. *Proc Natl Acad Sci U S A* (1999) **96**:9938–43. doi:10.1073/pnas.96.17.9938
126. Dupré SM, Burt DW, Talbot R, Downing A, Mouzaki D, Waddington D, et al. Identification of melatonin-regulated genes in the ovine pituitary pars tuberalis, a target site for seasonal hormone control. *Endocrinology* (2008) **149**:5527–39. doi:10.1210/en.2008-0834
127. Guilding C, Hughes AT, Brown TM, Namvar S, Piggins HD. A riot of rhythms: neuronal and glial circadian oscillators in the mediobasal hypothalamus. *Mol Brain* (2009) **2**:28. doi:10.1186/1756-6606-2-28
128. Dardente H, Menet JS, Poirel VJ, Streicher D, Gauer F, Vivien-Roels B, et al. Melatonin induces Cry1 expression in the pars tuberalis of the rat. *Brain Res Mol Brain Res* (2003) **114**:101–6. doi:10.1016/S0169-328X(03)00134-7
129. Hazlerigg DG, Andersson H, Johnston JD, Lincoln G. Molecular characterization of the long-day response in the Soay sheep, a seasonal mammal. *Curr Biol* (2004) **14**:334–9. doi:10.1016/j.cub.2004.01.057
130. Dardente H, Fortier EE, Martineau V, Cermakian N. Cryptochromes impair phosphorylation of transcriptional activators in the clock: a general mechanism for circadian repression. *Biochem J* (2007) **402**:525–36. doi:10.1042/BJ20060827
131. Ukai-Tadenuma M, Yamada RG, Xu H, Ripperger JA, Liu AC, Ueda HR. Delay in feedback repression by cryptochrome 1 is required for circadian clock function. *Cell* (2011) **144**:268–81. doi:10.1016/j.cell.2010.12.019
132. Maywood ES, Drynan L, Chesham JE, Edwards MD, Dardente H, Fustin JM, et al. Analysis of core circadian feedback loop in suprachiasmatic nucleus of mCry1-luc transgenic reporter mouse. *Proc Natl Acad Sci U S A* (2013) **110**:9547–52. doi:10.1073/pnas.1220894110
133. Fustin JM, Dardente H, Wagner GC, Carter DA, Johnston JD, Lincoln GA, et al. Egr1 involvement in evening gene regulation by melatonin. *FASEB J* (2009) **23**:764–73. doi:10.1096/fj.08-121467
134. Unfried C, Burbach G, Korf HW, von Gall C. Melatonin receptor 1-dependent gene expression in the mouse pars tuberalis as revealed by cDNA microarray analysis and in situ hybridization. *J Pineal Res* (2010) **48**:148–56. doi:10.1111/j.1600-079X.2009.00738.x
135. West A, Dupré SM, Yu L, Paton IR, Miedzinska K, McNeilly AS, et al. Npas4 is activated by melatonin, and drives the clock gene Cry1 in the ovine pars tuberalis. *Mol Endocrinol* (2013) **27**:979–89. doi:10.1210/me.2012-1366
136. Lincoln G, Messager S, Andersson H, Hazlerigg D. Temporal expression of seven clock genes in the suprachiasmatic nucleus and the pars tuberalis of the sheep: evidence for an internal coincidence timer. *Proc Natl Acad Sci U S A* (2002) **99**:13890–5. doi:10.1073/pnas.212517599
137. Yasuo S, Watanabe M, Tsukada A, Takagi T, Iigo M, Shimada K, et al. Photoinducible phase-specific light induction of Cry1 gene in the pars tuberalis of Japanese quail. *Endocrinology* (2004) **145**:1612–6. doi:10.1210/en.2003-1285
138. Dardente H, Wyse CA, Birnie MJ, Dupré SM, Loudon AS, Lincoln GA, et al. A molecular switch for photoperiod responsiveness in mammals. *Curr Biol* (2010) **20**:2193–8. doi:10.1016/j.cub.2010.10.048
139. Masumoto KH, Ukai-Tadenuma M, Kasukawa T, Nagano M, Uno KD, Tsujino K, et al. Acute induction of Eya3 by late-night light stimulation triggers TSHbeta expression in photoperiodism. *Curr Biol* (2010) **20**:2199–206. doi:10.1016/j.cub.2010.11.038
140. Tsujino K, Narumi R, Masumoto KH, Susaki EA, Shinohara Y, Abe T, et al. Establishment of TSH beta real-time monitoring system in mammalian photoperiodism. *Genes Cells* (2013) **18**:575–88. doi:10.1111/gtc.12063
141. Helfer G, Ross AW, Russell L, Thomson LM, Shearer KD, Goodman TH, et al. Photoperiod regulates vitamin A and Wnt/beta-catenin signaling in F344 rats. *Endocrinology* (2012) **153**:815–24. doi:10.1210/en.2011-1792
142. Ross AW, Bell LM, Littlewood PA, Mercer JG, Barrett P, Morgan PJ. Temporal changes in gene expression in the arcuate nucleus precede seasonal responses in adiposity and reproduction. *Endocrinology* (2005) **146**:1940–7. doi:10.1210/en.2004-1538
143. Barrett P, Ross AW, Balik A, Littlewood PA, Mercer JG, Moar KM, et al. Photoperiodic regulation of histamine H3 receptor and VGF messenger ribonucleic acid in the arcuate nucleus of the Siberian hamster. *Endocrinology* (2005) **146**:1930–9. doi:10.1210/en.2004-1452
144. Klosen P, Sebert ME, Rasri K, Laran-Chich MP, Simonneaux V. TSH restores a summer phenotype in photoinhibited mammals via the RF-amides RFRP3 and kisspeptin. *FASEB J* (2013) **27**:2677–86. doi:10.1096/fj.13-229559
145. Morgan PJ, Hazlerigg DG. Photoperiodic signalling through the melatonin receptor turns full circle. *J Neuroendocrinol* (2008) **20**:820–6. doi:10.1111/j.1365-2826.2008.01724.x
146. Shearer KD, Goodman TH, Ross AW, Reilly L, Morgan PJ, McCaffery PJ. Photoperiodic regulation of retinoic acid signaling in the hypothalamus. *J Neurochem* (2010) **112**:246–57. doi:10.1111/j.1471-4159.2009.06455.x
147. Bechtold DA, Sidibe A, Saer BR, Li J, Hand LE, Ivanova EA, et al. A role for the melatonin-related receptor GPR50 in leptin signaling, adaptive thermogenesis, and torpor. *Curr Biol* (2012) **22**:70–7. doi:10.1016/j.cub.2011.11.043
148. Hand LE, Saer BR, Hui ST, Jinnah HA, Steinlechner S, Loudon AS, et al. Induction of the metabolic regulator Txnip in fasting-induced and natural torpor. *Endocrinology* (2013) **154**:2081–91. doi:10.1210/en.2012-2051
149. Scherbarth F, Steinlechner S. Endocrine mechanisms of seasonal adaptation in small mammals: from early results to present understanding. *J Comp Physiol B* (2010) **180**:935–52. doi:10.1007/s00360-010-0498-2
150. Bolborea M, Dale N. Hypothalamic tanycytes: potential roles in the control of feeding and energy balance. *Trends Neurosci* (2013) **36**:91–100. doi:10.1016/j.tins.2012.12.008
151. Paul MJ, Zucker I, Schwartz WJ. Tracking the seasons: the internal calendars of vertebrates. *Philos Trans R Soc Lond B Biol Sci* (2008) **363**:341–61. doi:10.1098/rstb.2007.2143
152. Follett BK, Nicholls TJ. Photorefractoriness in Japanese quail: possible involvement of the thyroid gland. *J Exp Zool* (1984) **232**:573–80. doi:10.1002/jez.1402320325
153. Hazlerigg DG, Lincoln GA. Hypothesis: cyclical histogenesis is the basis of circannual timing. *J Biol Rhythms* (2011) **26**:471–85. doi:10.1177/0748730411420812

154. Migaud M, Batailler M, Pillon D, Franceschini I, Malpaux B. Seasonal changes in cell proliferation in the adult sheep brain and pars tuberalis. *J Biol Rhythms* (2011) **26**:486–96. doi:10.1177/0748730411420062
155. Lee DA, Blackshaw S. Functional implications of hypothalamic neurogenesis in the adult mammalian brain. *Int J Dev Neurosci* (2012) **30**:615–21. doi:10.1016/j.ijdevneu.2012.07.003
156. Hazlerigg DG, Wyse CA, Dardente H, Hanon EA, Lincoln GA. Photoperiodic variation in CD45-positive cells and cell proliferation in the mediobasal hypothalamus of the Soay sheep. *Chronobiol Int* (2013) **30**:548–58. doi:10.3109/07420528.2012.754450
157. Saenz de Miera C, Hanon EA, Dardente H, Birnie M, Simonneaux V, Lincoln GA, et al. Circannual variation in thyroid hormone deiodinases in a short-day breeder. *J Neuroendocrinol* (2013) **25**:412–21. doi:10.1111/jne.12013
158. Murphy M, Ebling FJ. The role of hypothalamic tri-iodothyronine availability in seasonal regulation of energy balance and body weight. *J Thyroid Res* (2011) **2011**:387562. doi:10.4061/2011/387562
159. Simonneaux V, Bur I, Ancel C, Ansel L, Klosen P. A kiss for daily and seasonal reproduction. *Prog Brain Res* (2012) **199**:423–37. doi:10.1016/B978-0-444-59427-3.00024-1
160. Clarke IJ, Caraty A. Kisspeptin and seasonality of reproduction. *Adv Exp Med Biol* (2013) **784**:411–30. doi:10.1007/978-1-4614-6199-9_19
161. Beltramo M, Dardente H, Cayla X, Caraty A. Cellular mechanisms and integrative timing of neuroendocrine control of GnRH secretion by kisspeptin. *Mol Cell Endocrinol* (2014) **382**:387–99. doi:10.1016/j.mce.2013.10.015
162. Oakley AE, Clifton DK, Steiner RA. Kisspeptin signaling in the brain. *Endocr Rev* (2009) **30**:713–43. doi:10.1210/er.2009-0005
163. Hill JW, Alreja M, Elias CF. From precocious puberty to infertility: metabolic control of the reproductive function. *Front Endocrinol (Lausanne)* (2013) **4**:43. doi:10.3389/fendo.2013.00043
164. Ebling FJ, Foster DL. Pineal melatonin rhythms and the timing of puberty in mammals. *Experientia* (1989) **45**:946–54. doi:10.1007/BF01953052
165. Ebling FJ. Photoperiodic regulation of puberty in seasonal species. *Mol Cell Endocrinol* (2010) **324**:95–101. doi:10.1016/j.mce.2010.03.018
166. Clarke IJ, Smith JT, Henry BA, Oldfield BJ, Stefanidis A, Millar RP, et al. Gonadotropin-inhibitory hormone is a hypothalamic peptide that provides a molecular switch between reproduction and feeding. *Neuroendocrinology* (2012) **95**:305–16. doi:10.1159/000332822
167. Kirby ED, Geraghty AC, Ubuka T, Bentley GE, Kaufer D. Stress increases putative gonadotropin inhibitory hormone and decreases luteinizing hormone in male rats. *Proc Natl Acad Sci U S A* (2009) **106**:11324–9. doi:10.1073/pnas.0901176106
168. Clarke IJ, Sari IP, Qi Y, Smith JT, Parkington HC, Ubuka T, et al. Potent action of RFamide-related peptide-3 on pituitary gonadotropes indicative of a hypophysiotropic role in the negative regulation of gonadotropin secretion. *Endocrinology* (2008) **149**:5811–21. doi:10.1210/en.2008-0575
169. Caraty A, Blomenrohr M, Vogel GM, Lomet D, Briant C, Beltramo M. RF9 powerfully stimulates gonadotropin secretion in the ewe: evidence for a seasonal threshold of sensitivity. *J Neuroendocrinol* (2012) **24**:725–36. doi:10.1111/j.1365-2826.2012.02283.x
170. Ancel C, Bentsen AH, Sebert ME, Tena-Sempere M, Mikkelsen JD, Simonneaux V. Stimulatory effect of RFRP-3 on the gonadotropic axis in the male Syrian hamster: the exception proves the rule. *Endocrinology* (2012) **153**:1352–63. doi:10.1210/en.2011-1622
171. Ubuka T, Inoue K, Fukuda Y, Mizuno T, Ukena K, Kriegsfeld LJ, et al. Identification, expression, and physiological functions of Siberian hamster gonadotropin-inhibitory hormone. *Endocrinology* (2012) **153**:373–85. doi:10.1210/en.2011-1110
172. Tsutsui K, Ubuka T, Bentley GE, Kriegsfeld LJ. Review: regulatory mechanisms of gonadotropin-inhibitory hormone (GnIH) synthesis and release in photoperiodic animals. *Front Neurosci* (2013) **7**:60. doi:10.3389/fnins.2013.00060
173. Osugi T, Ohtaki N, Sunakawa Y, Son YL, Ohkubo M, Iigo M, et al. Molecular evolution of kiss2 genes and peptides in vertebrates. *Endocrinology* (2013) **154**:4270–80. doi:10.1210/en.2012-2267
174. Kriegsfeld LJ. Driving reproduction: RFamide peptides behind the wheel. *Horm Behav* (2006) **50**:655–66. doi:10.1016/j.yhbeh.2006.06.004
175. Simonneaux V, Ansel L, Revel FG, Klosen P, Pevet P, Mikkelsen JD. Kisspeptin and the seasonal control of reproduction in hamsters. *Peptides* (2009) **30**:146–53. doi:10.1016/j.peptides.2008.06.006
176. Henson JR, Carter SN, Freeman DA. Exogenous T(3) elicits long day-like alterations in testis size and the RFamides kisspeptin and gonadotropin-inhibitory hormone in short-day Siberian hamsters. *J Biol Rhythms* (2013) **28**:193–200. doi:10.1177/0748730413487974

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Regulation of seasonal reproduction by hypothalamic activation of thyroid hormone

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Organisms living outside the tropics measure the changes in the length of the day to adapt to seasonal changes in the environment. Animals that breed during spring and summer are called long-day breeders, while those that breed during fall are called short-day breeders. Although the influence of thyroid hormone in the regulation of seasonal reproduction has been known for several decades, its precise mechanism remained unknown. Recent studies revealed that the activation of thyroid hormone within the mediobasal hypothalamus plays a key role in this phenomenon. This localized activation of the thyroid hormone is controlled by thyrotropin (thyroid-stimulating hormone) secreted from the pars tuberalis of the pituitary gland. Although seasonal reproduction is a rate-limiting factor in animal production, genes involved in photoperiodic signal transduction pathway could emerge as potential targets to facilitate domestication.

Keywords: seasonal reproduction, mediobasal hypothalamus, ependymal cell, pars tuberalis, thyrotropin, thyroid hormone, iodothyronine deiodinase

INTRODUCTION

Orbiting of the earth around the sun causes changing seasons. To adapt to the seasonal changes in the environment, animals alter their physiology and behavior, which is characterized by the changes in growth, metabolism, immune function, reproductive activity, migration, hibernation, and molting. Most of the organisms use the changes in the length of the day (photoperiod) as a calendar, because temperature and precipitation varies throughout each year and are unreliable when compared with the length of the day. This phenomenon is called "photoperiodism" (1). Among the various seasonally regulated phenomena, the mechanism of seasonal reproduction has been extensively studied. Small mammals and birds breed during the spring and summer. Therefore, they are called long-day (LD) breeders. The gestation or incubation period of these animals last only a few weeks and their offspring are born during the spring and summer. In contrast, larger mammals, such as goats and sheep, breed during fall. Therefore, they are called short-day (SD) breeders. These animals have a gestation period of approximately 6 months. Therefore, their offspring are also born and raised during spring and summer. Accordingly, the offspring of both LD and SD breeders grow when the climate is moderate and food is abundant (**Figure 1**).

Seasonal reproduction of vertebrate species is regulated by the hypothalamic–pituitary–gonadal (HPG) axis. The secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus induces the secretion of gonadotropins [luteinizing hormone (LH) and follicle-stimulating hormone (FSH)] from the anterior pituitary gland, which in turn activates gonadal activity. In other words, the HPG axis of seasonally breeding animals is only activated during the breeding season. Among the various vertebrate species, birds show the most dramatic changes in gonadal size (typically more than a 100-fold) (2). Therefore, birds have a highly sophisticated photoperiodic mechanism in comparison to other vertebrate species (3). In addition to the robust gonadal responses, most of the birds have very short breeding seasons, as the HPG axis is automatically switched off and their gonads start to regress even though the length of the day is still increasing. This phenomenon is known as photorefractoriness (4, 5). The length of the breeding season tends to be shorter in higher latitude due to the short benign season in higher latitude. Among mammals, hamsters and sheep are extensively studied, because they show dramatic photoperiodic responses. However, the magnitude of the seasonal gonadal development and regression is less robust in mammals than in birds, as their gonads change only by a few-folds.

INFLUENCE OF THYROID HORMONE IN THE SEASONAL CHANGES

It has been known for many decades that thyroid hormone is somehow involved in the regulation of seasonal reproductive function in various organisms including fish, birds, and mammals (2, 6, 7). In some species, thyroidectomy prevents the transition to reproductive state (i.e., seasonal testicular development and/or regression) (8–11), and thyroxine (T_4) treatment mimics the effects of a long photoperiod (12–14). However, photo-stimulated gonadal

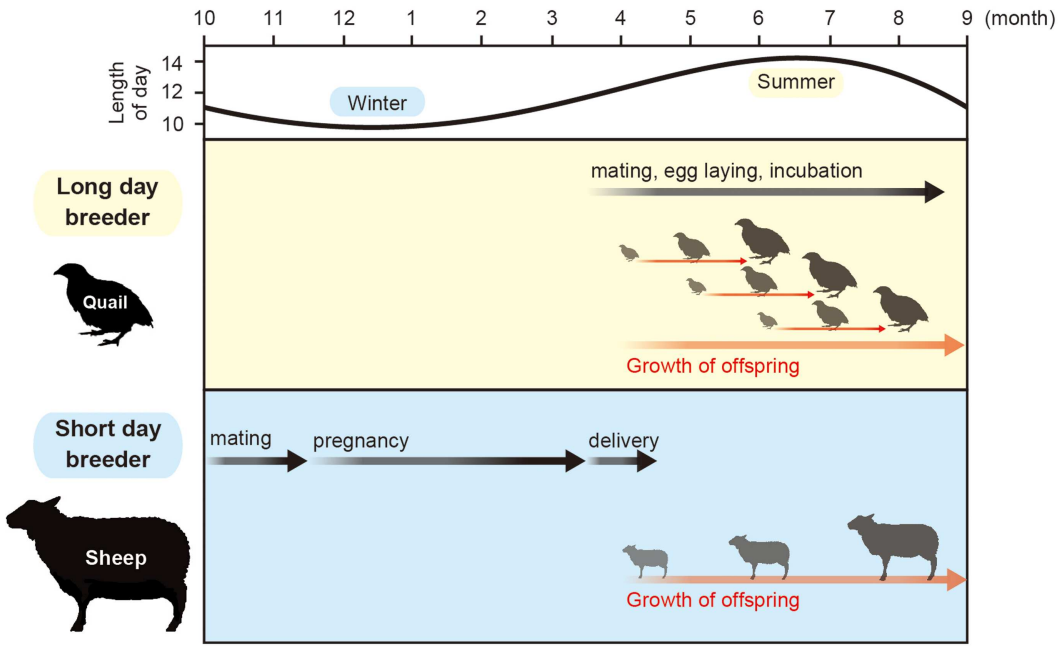

FIGURE 1 | Calendar of seasonal breeding animals. Most animals mate in a specific time of a year. Small animals with short gestation or incubation period mate in spring and summer, while large animals that have a 6-month gestation period mate in fall to give birth in spring.

maturation appears to have been largely unaffected by thyroidectomy in some species (2). Therefore, the reported effects of thyroidectomy on seasonal breeding are often contradictory and the role of T_4 is thought to be permissive. Although the requirement of T_4 for an appropriate response to photoperiod has been documented (15), the mechanism by which thyroid hormone regulates seasonal reproduction remained unknown for several decades.

PHOTOPERIODIC CHANGES IN TYPE 2 AND TYPE 3 DEIODINASES WITHIN THE HYPOTHALAMUS

The Japanese quail (*Coturnix japonica*) is an excellent model for studying photoperiodism, because of its rapid and robust responses to changing photoperiods (3). Local illumination of the mediobasal hypothalamus (MBH) by radioluminous-painted beads induce testicular growth (16), and lesions of MBH blocks the photoperiodic response of LH secretion and gonadal development (17, 18). In addition, expression of c-Fos, a marker of neuronal activation, is induced in the MBH by LD stimulus (19). The MBH is therefore considered central for the seasonal reproduction in quail. By using differential subtractive hybridization analysis, LD-induction of type 2 deiodinase gene (*DIO2*) and LD-suppression of type 3 deiodinase gene (*DIO3*) were observed in the ependymal cells (also known as tanycytes) that line the ventrolateral walls of the third ventricle within the MBH [Ref. (20, 21), **Figure 2**]. *DIO2* encodes the thyroid hormone-activating enzyme that converts the prohormone T_4 to bioactive triiodothyronine (T_3) (22), while *DIO3* encodes thyroid hormone-inactivating enzyme that metabolizes T_4 and T_3 to inactive reverse T_3 (rT_3) and 3,3′-diiodothyronine (T_2), respectively. The reciprocal switching of *DIO2* and *DIO3* appears to regulate the local thyroid hormone concentration precisely within the MBH. Moreover, T_3 concentration within the MBH is about 10-fold higher under LD conditions than under SD conditions, even though plasma concentrations are similar to both photoperiods (20). The functional significance of this locally activated thyroid hormone has been demonstrated by pharmacological analyses. Intracerebroventricular (i.c.v.) infusion of T_3 in SD conditions induced testicular development while infusion of a DIO2 inhibitor (iopanoic acid) in LD conditions attenuated testicular development (20). Photoperiodic regulation of *DIO2* and/or *DIO3* has also been confirmed in a number of other avian species, such as the tree sparrow (23), chicken (24), great tits (25), and canary (26). Similarly, photoperiodic regulation of thyroid hormone metabolism in the MBH has been confirmed in various mammalian species, including LD breeders like Siberian hamsters (27–30), Syrian hamsters (31, 32), rats (33, 34), mice (35), and SD-breeding goats (36) and sheep (37). Activation of thyroid hormone within the MBH decodes the LD information. Therefore, daily T_3 subcutaneous injections induce testicular development (28) and chronic replacement of T_3 in the hypothalamus prevents the onset of testicular regression (27) in LD-breeding Siberian hamsters. In contrast, in the SD breeders, LD-induced DIO2 appears to convert T_4 to T_3 to terminate the breeding season (37). In addition, LD stimulus induces the expression of *DIO2*, and T_4 administration terminates the breeding season via a decrease in serum LH (38, 39).

THYROID HORMONE TRANSPORT TO THE EPENDYMAL CELLS

Due to their lipophilic nature, thyroid hormones are believed to traverse plasma membranes by passive diffusion. However,

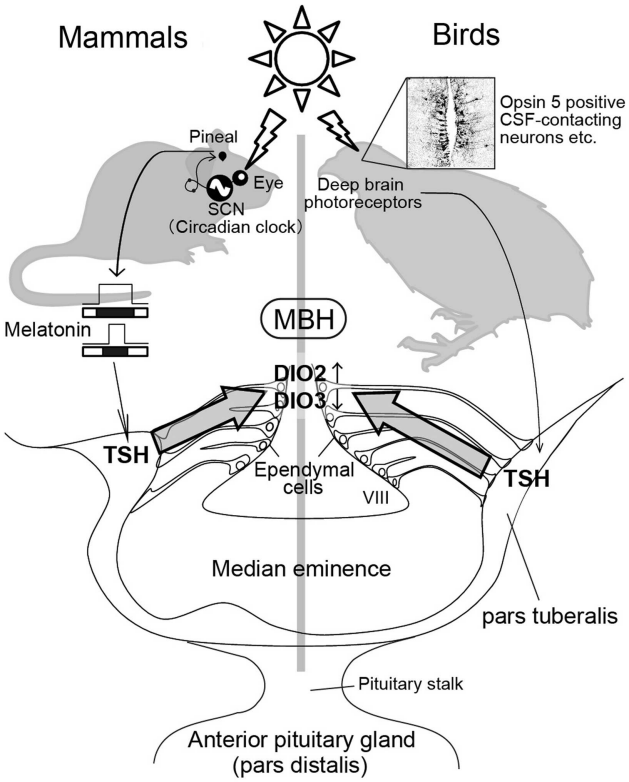

FIGURE 2 | Photoperiodic signal transduction pathway in mammals and birds. In mammals, light information is received by the eye and transmitted to the pineal gland via the circadian pacemaker, the suprachiasmatic nucleus (SCN). The duration of the pineal melatonin signal encodes the length of night and regulates TSH secretion in the pars tuberalis. The pars tuberalis TSH acts on TSH receptor expressed in the ependymal cells lining ventrolateral walls of the third ventricle (VIII) to induce DIO2 and reduce DIO3. Local thyroid hormone activation within the mediobasal hypothalamus (MBH) by DIO2/DIO3 switching plays a key role in the regulation of seasonal reproduction. In contrast, light information received by deep brain photoreceptors induces TSH secretion from the pars tuberalis in birds. Nevertheless, melatonin is not involved in the seasonal reproduction of birds. The schematic is a modified version of illustration published by Ikegami and Yoshimura (40).

involvement of a membrane transport system for thyroid hormone has been reported recently and a mechanism that facilitates the transport of thyroid hormone into the ependymal cells was examined. Some members of the organic anion transporting polypeptide (Oatp) family have been shown to transport thyroid hormones in mammals (41, 42) and the involvement of a member of this family in transporting T_4 into the quail brain has been investigated (43). Oatp1c1, which is expressed in the ependymal cells within the MBH, has been demonstrated to be a highly specific transporter of T_4. In addition to Oatp1c1, another thyroid hormone transporter, monocarboxylate transporter 8 (MCT8), has been found in the ependymal cells within hamster MBH (29). Although MCT8 appears to be involved in the regulation of photoperiodism, its expression is upregulated under SD conditions, which does not require thyroid hormone.

REGULATION OF HYPOTHALAMIC DEIODINASES BY THE PARS TUBERALIS TSH

When quail are transferred from SD conditions to LD conditions, an increase in plasma gonadotropin (LH) is observed 22 h after the dawn of the first LD (3, 44, 45). As discussed previously, reciprocal switching of *DIO2* and *DIO3* plays a critical role in the regulation of seasonal reproduction in birds and mammals. In quail, the reciprocal switching of *DIO2* and *DIO3* precedes photoperiodic induction of gonadotropin release by roughly 4 h (21). Genome-wide gene expression analysis during the transition from SD conditions to LD conditions in Japanese quail (45) identified the induction of two genes 4 h prior to *DIO2*/*DIO*3 switching (i.e., 14 h after dawn) in the pars tuberalis of the pituitary gland. The pars tuberalis consists of thin layers of cells surrounding the median eminence (**Figure 2**). One of these genes encode the thyroid-stimulating hormone β subunit (*TSHB*) and the other encode the transcriptional co-activator eyes absent 3 (*EYA3*). Although EYA3 is a transcriptional co-activator, the expression sites of *EYA3* and *DIO2*/*DIO3* are different (i.e., *EYA3* in the pars tuberalis and *DIO2/DIO3* in the ependymal cells). Therefore, it appears that EYA3 is not involved in the regulation of *DIO2*/*DIO3* switching. On the other hand, the expression of TSH receptor (TSHR) and binding of ^{125}I-labeled thyroid-stimulating hormone (TSH) were observed in the ependymal cells where *DIO2* and *DIO3* are expressed. In addition to these, i.c.v. TSH administration induced *DIO2* expression and reduced *DIO3* expression in the ependymal cells even under SD conditions, while passive immunization against TSH attenuated LD-induction of *DIO2* expression (45). The involvement of TSHR-Gsα-cAMP signaling pathway in this TSH regulation of *DIO2* expression was demonstrated by the promoter analysis. Considering that the magnitude of testicular growth induced by i.c.v. TSH infusion was almost similar to that observed in birds exposed to LD stimulus, the LD-induced pars tuberalis TSH appears to be a major factor regulating the seasonal reproduction in birds.

In birds, eyes are not necessary for the regulation of seasonal reproduction because deep brain photoreceptors are involved in this process (46, 47). Although pineal organ is a photoreceptive organ in non-mammalian vertebrates (48, 49), pineal organ is not involved in the regulation of seasonal reproduction (50, 51). In contrast, local illumination of the septal region of the telencephalon or the MBH using radioluminous-painted beads caused testicular growth in quail, suggesting the existence of deep brain photoreceptors in these regions (16). Localization of several rhodopsin family proteins (rhodopsin; OPN4: melanopsin; OPN5: neuropsin and VA opsin: vertebrate ancient opsin) are reported in these brain regions and projections that link some of these photoreceptor cells to the pars tuberalis have also been reported (52–62). These photoreceptors are therefore thought to be involved in the seasonal regulation of reproduction in birds (**Figure 2**).

In a marked contrast to avian species, eyes are the only photoreceptive organ in mammalian species (63–69). Therefore, removal of the eyes abolishes the photoperiodic response (64, 68). Light information received by the eye is transmitted to the pineal gland through the suprachiasmatic nucleus (SCN), where the circadian

pacemaker is localized (68, 70–74). The duration of night corresponds to the nocturnal secretion profile of melatonin, which plays a crucial role in the regulation of seasonal reproduction in mammalian species. For example, in both LD and SD breeders, pinealectomy abolishes seasonal responses, while melatonin administration restores them (68, 74, 75). Melatonin acts via melatonin receptors and there are two subtypes of melatonin receptors (MT1 and MT2) in mammals (76, 77). However, these melatonin receptors are not expressed in the ependymal cells where *DIO2* and *DIO3* are expressed (78, 79). The MT1 receptor is reportedly expressed in the thyrotroph cells of the pars tuberalis (80, 81). Therefore, pars tuberalis TSH likely mediates the influence of melatonin in the *DIO2*/*DIO3* switching in mammalian species. Although it is generally considered that laboratory mice are non-seasonal breeders, many researchers noticed that mice do not breed well during the winter (e.g., small litter size) even though they are kept under standardized conditions. To determine whether pars tuberalis TSH mediates the influence of melatonin in the *DIO2/DIO3* switching, laboratory mice were analyzed as experimental models. Two key enzymes, arylalkylamine *N*-acetyltransferase (AA-NAT) and hydroxyindole-*O*-methyltransferase (HIOMT) are involved in melatonin biosynthesis from serotonin (74). However, most inbred mice genetically lack the ability to produce these enzymes, resulting in minimal melatonin generation (82, 83). Therefore, it was predicted that melatonin-producing strains would have the capacity to respond to photoperiodic changes, while melatonin-deficient strains would be resilient to such changes. As expected, clear photoperiodic regulation of *TSHB*, *DIO2*, and *DIO3* was observed in the melatonin-producing CBA strain, while such responses were not observed in the melatonin-deficient C57BL strain (35). In addition, daily intraperitoneal (i.p.) melatonin injections mimicked the effect of SD conditions on the expression of these genes (35). To test the involvement of the TSH–TSHR signaling pathway in the melatonin-mediated regulation of *DIO2*/*DIO3* expression, the effects of melatonin administration were examined in TSHR-null mice (35). The TSHR-null mice failed to respond to melatonin administration. This result clearly suggested the involvement of a TSH–TSHR signaling pathway in the melatonin-mediated regulation of *DIO2*/*DIO3* in mammals. In addition, the analysis of mice that lacked the MT1 and MT2 melatonin receptors revealed the involvement of MT1 melatonin receptors in this regulation (84). It is also interesting to note that TSH is involved in the LD-induction of *DIO2* in SD-breeding sheep (37). Thus, pars tuberalis TSH appears to relay the seasonal information in both LD and SD-breeding animals and sensitize them for spring.

THYROID HORMONE ACTION WITHIN THE HYPOTHALAMUS

Thyroid hormone is involved in the development and plasticity of the central nervous system (22). The expression of thyroid hormone receptors (*THR*α, *THR*β, and *RXR*α) in the median eminence suggested that the median eminence is the target site of action for the photo-induced increase in T_3 in the quail MBH (20). To understand the action of thyroid hormone within the MBH, the ultrastructure of the median eminence was examined under SD and LD conditions using electron microscopy. Dynamic morphological changes were observed between the GnRH nerve terminals and glial endfeet within the median eminence (85). In SD conditions, many GnRH nerve terminals are encased by the endfeet of glial processes and do not contact the basal lamina, while many GnRH nerve terminals are in close proximity to the basal lamina under LD conditions (**Figure 3**). It has been proposed that the nerve terminals of hypothalamic neurons are required to directly contact the pericapillary space for the secretion of the hypothalamic neurohormone from the hypothalamus into the portal capillary (86). Morphological changes between the GnRH nerve terminals and endfeet of glial processes are observed in SD quail treated with T_3 to stimulate testicular growth (87). Therefore, these morphological changes appear to regulate or modulate the seasonal GnRH secretion from the median eminence. It is also interesting to note that the seasonal plasticity within the GnRH system is reported in ewes (88).

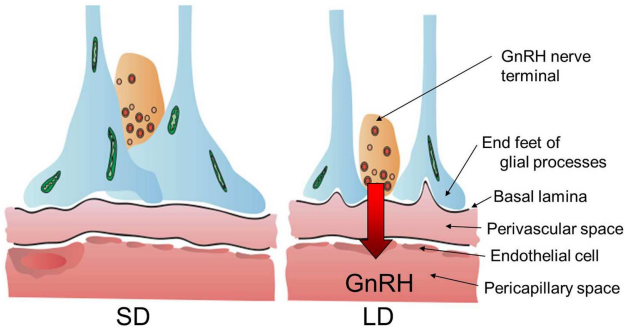

FIGURE 3 | Neuro–glial interaction between GnRH nerve terminals and glial endfeet. Locally activated thyroid hormone within the MBH regulates neuro–glial interaction in the median eminence and these morphological changes appear to regulate or modulate seasonal GnRH secretion from the hypothalamus to portal capillary. The illustration has been modified from that published by Yoshimura (89).

PHOTOPERIODIC SIGNALING PATHWAY AND DOMESTICATION

Seasonal reproduction is a rate-limiting factor for the animal procreation. The photoperiodic signaling pathway could also be a potential target that facilitates human-driven domestication process. As discussed previously, most laboratory mice lack the enzyme activity of melatonin biosynthesis pathway (82, 83, 90, 91). In addition, occurrence of selective sweeps was found at the TSHR locus in all domestic chickens (92). This observation suggests that the TSHR may be a domestication locus in chicken (92). Although we still do not know the correlation with domestication, it is interesting to note that photoperiodic regulation of *DIO3* is absent in Syrian hamster (27). Thus, genes involved in the photoperiodic signaling pathway could emerge as useful targets for the domestication of wild animals.

CONCLUSION

Involvement of thyroid hormone in the regulation of seasonal reproduction has been suggested in the past several decades. Recent comparative studies clearly reveal that the local activation of thyroid hormone within the hypothalamus is a key factor in the

regulation of seasonal reproduction in a number of mammalian and avian species. It is important to note that this mechanism is also conserved in fish (93) and is universal among various vertebrate species. Although thyroid hormone influences both LD and SD breeders, the mechanism that differentiates LD breeders from SD breeders remains unknown. Presumably, the responsiveness of pathways downstream of T_3 activity (e.g., responsiveness of T_3 target genes to LD-induced T_3 etc.) differs in LD and SD breeders. The switching mechanism of LD breeder and SD breeder needs to be clarified in the future studies.

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REFERENCES

1. Garner WW, Allard HA. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. *J Agric Res* (1920) **18**:553–606.
2. Dawson A, King VM, Bentley GE, Ball GF. Photoperiodic control of seasonality in birds. *J Biol Rhythms* (2001) **16**:365–80. doi:10.1177/074873001129002079
3. Follett BK, King VM, Meddle SL. Rhythms and photoperiodism in birds. In: Lumsden PJ, Miller AJ editors. *Biological Rhythms and Photoperiodism in Plants.* Oxford: Biostatistics Scientific (1998). p. 231–42.
4. Hahn TP, MacDougall-Shackleton SA. Adaptive specialization, conditional plasticity and phylogenetic history in the reproductive cue response systems of birds. *Philos Trans R Soc Lond B Biol Sci* (2008) **363**:267–86. doi:10.1098/rstb.2007.2139
5. Nicholls TJ, Goldsmith AR, Dawson A. Photorefractoriness in birds and comparison with mammals. *Physiol Rev* (1988) **68**:133–76.
6. Cyr DG, Eales JG. Interrelationships between thyroidal and reproductive endocrine systems in fish. *Rev Fish Biol Fish* (1996) **6**:165–200. doi:10.1007/BF00182342
7. Nicholls TJ, Follett BK, Goldsmith AR, Pearson H. Possible homologies between photorefractoriness in sheep and birds: the effect of thyroidectomy on the length of the ewe's breeding season. *Reprod Nutr Dev* (1988) **28**:375–85. doi:10.1051/rnd:19880304
8. Moenter SM, Woodfill CJ, Karsch FJ. Role of the thyroid gland in seasonal reproduction: thyroidectomy blocks seasonal suppression of reproductive neuroendocrine activity in ewes. *Endocrinology* (1991) **128**:1337–44. doi:10.1210/endo-128-3-1337
9. Dawson A. Thyroidectomy progressively renders the reproductive system of starlings (*Sturnus vulgaris*) unresponsive to changes in daylength. *J Endocrinol* (1993) **139**:51–5. doi:10.1677/joe.0.1390051
10. Dawson A. Thyroidectomy of house sparrows (*Passer domesticus*) prevents photo-induced testicular growth but not the increased hypothalamic gonadotrophin-releasing hormone. *Gen Comp Endocrinol* (1998) **110**:196–200. doi:10.1006/gcen.1998.7065
11. Parkinson TJ, Follett BK. Thyroidectomy abolishes seasonal testicular cycles of Soay rams. *Proc Biol Sci* (1995) **259**:1–6. doi:10.1098/rspb.1995.0001
12. Follett BK, Nicholls TJ. Influences of thyroidectomy and thyroxine replacement on photoperiodically controlled reproduction in quail. *J Endocrinol* (1985) **107**:211–21. doi:10.1677/joe.0.1070211
13. Goldsmiths AR, Nicholls TJ. Thyroxine effects upon reproduction, prolactin secretion and plumage moult in thyroidectomised European starlings *Sturnus vulgaris*. *Ornis Scand* (1992) **23**:398–404. doi:10.2307/3676666
14. Wilson FE, Reinert BD. Thyroid hormone acts centrally to programme photostimulated male American tree sparrows (*Spizella arborea*) for vernal and autumnal components of seasonality. *J Neuroendocrinol* (2000) **12**:87–95. doi:10.1046/j.1365-2826.2000.00437.x
15. Bentley GE. Photoperiodism and reproduction in birds. In: Nelson RJ, Denlinger DL, Somers DE editors. *Photoperiodism: The Biological Calendar.* New York: Oxford University Press (2010). p. 420–45.
16. Homma K, Ohta M, Sakakibara Y. Photoinducible phase of the Japanese quail detected by direct stimulation of the brain. In: Suda M, Hayaishi O, Nakagawa H editors. *Biological Rhythms and Their Central Mechanism.* Amsterdam: Elsevier (1979). p. 85–94.
17. Sharp PJ, Follett BK. The effect of hypothalamic lesions on gonadotrophin release in Japanese quail (*Coturnix coturnix japonica*). *Neuroendocrinology* (1969) **5**:205–18. doi:10.1159/000121861
18. Juss TS, Meddle SL, Servant RS, King VM. Melatonin and photoperiodic time measurement in Japanese quail (*Coturnix coturnix japonica*). *Proc R Soc Lond B Biol Sci* (1993) **254**:21–8. doi:10.1098/rspb.1993.0121
19. Meddle SL, Follett BK. Photoperiodically driven changes in Fos expression within the basal tuberal hypothalamus and median eminence of Japanese quail. *J Neuroscience* (1997) **17**:8909–18.
20. Yoshimura T, Yasuo S, Watanabe M, Iigo M, Yamamura T, Hirunagi K, et al. Light-induced hormone conversion of T_4 to T_3 regulates photoperiodic response of gonads in birds. *Nature* (2003) **426**:178–81. doi:10.1038/nature02117
21. Yasuo S, Watanabe M, Nakao N, Takagi T, Follett BK, Ebihara S, et al. The reciprocal switching of two thyroid hormone-activating and – inactivating enzyme genes is involved in the photoperiodic gonadal response of Japanese quail. *Endocrinology* (2005) **146**:2551–4. doi:10.1210/en.2005-0057
22. Bernal J. Action of thyroid hormone in brain. *J Endocrinol Invest* (2002) **25**:268–88.
23. Watanabe T, Yamamura T, Watanabe M, Yasuo S, Nakao N, Dawson A, et al. Hypothalamic expression of thyroid hormone-activating and -inactivating enzyme genes in relation to photorefractoriness in birds and mammals. *Am J Physiol Regul Integr Comp Physiol* (2007) **292**:R568–72. doi:10.1152/ajpregu.00521.2006
24. Ono H, Nakao N, Yamamura T, Kinoshita K, Mizutani M, Namikawa T, et al. Red jungle fowl (*Gallus gallus*) as a model for studying the molecular mechanism of seasonal reproduction. *Anim Sci J* (2009) **80**:328–32. doi:10.1111/j.1740-0929.2009.00628.x
25. Perfito N, Jeong SY, Silverin B, Calisi RM, Bentley GE, Hau M. Anticipating spring: wild populations of great tits *(Parus major)* differ in expression of key genes for photoperiodic time measurement. *PLoS One* (2012) **7**:e34997. doi:10.1371/journal.pone.0034997
26. Stevenson TJ, Ball GF. Disruption of neuropsin mRNA expression via RNA interference facilitates the photoinduced increase in thyrotropin-stimulating subunit β in birds. *Eur J Neurosci* (2012) **36**:2859–65. doi:10.1111/j.1460-9568.2012.08209.x
27. Barrett P, Ebling FJ, Schuhler S, Wilson D, Ross AW, Warner A, et al. Hypothalamic thyroid hormone catabolism acts as a gatekeeper for the seasonal control of body weight and reproduction. *Endocrinology* (2007) **148**:3608–17. doi:10.1210/en.2007-0316
28. Freeman DA, Teubner BJ, Smith CD, Prendergast BJ. Exogenous T_3 mimics long day lengths in Siberian hamsters. *Am J Physiol Regul Integr Comp Physiol* (2007) **292**:R2368–72. doi:10.1152/ajpregu.00713.2006
29. Herwig A, Wilson D, Logie TJ, Boelen A, Morgan PJ, Mercer JG, et al. Photoperiod and acute energy deficits interact on components of the thyroid hormone system in hypothalamic tanycytes of the Siberian hamster. *Am J Physiol Regul Integr Comp Physiol* (2009) **296**:R1307–15. doi:10.1152/ajpregu.90755.2008
30. Watanabe M, Yasuo S, Watanabe T, Yamamura T, Nakao N, Ebihara S, et al. Photoperiodic regulation of type 2 deiodinase gene in Djungarian hamster: possible homologies between avian and mammalian photoperiodic regulation of reproduction. *Endocrinology* (2004) **145**:1546–9. doi:10.1210/en.2003-1593
31. Revel FG, Saboureau M, Pévet P, Mikkelsen JD, Simonneaux V. Melatonin regulates type 2 deiodinase gene expression in the Syrian hamster. *Endocrinology* (2006) **147**:4680–7. doi:10.1210/en.2006-0606
32. Yasuo S, Yoshimura T, Ebihara S, Korf HW. Temporal dynamics of type 2 deiodinase expression after melatonin injections in Syrian hamsters. *Endocrinology* (2007) **148**:4385–92. doi:10.1210/en.2007-0497
33. Ross AW, Helfer G, Russell L, Darras VM, Morgan PJ. Thyroid hormone signalling genes are regulated by photoperiod in the hypothalamus of F344 rats. *PLoS One* (2011) **6**:e21351. doi:10.1371/journal.pone.0021351
34. Yasuo S, Watanabe M, Iigo M, Nakamura TJ, Watanabe T, Takagi T, et al. Differential response of type 2 deiodinase gene expression to photoperiod between photoperiodic Fischer 344 and nonphotoperiodic Wistar rats. *Am J Physiol Regul Integr Comp Physiol* (2007) **292**:R1315–9. doi:10.1152/ajpregu.00396.2006

35. Ono H, Hoshino Y, Yasuo S, Watanabe M, Nakane Y, Murai A, et al. Involvement of thyrotropin in photoperiodic signal transduction in mice. *Proc Natl Acad Sci USA* (2008) **105**:18238–42. doi:10.1073/pnas.0808952105
36. Yasuo S, Nakao N, Ohkura S, Iigo M, Hagiwara S, Goto A, et al. Long-day suppressed expression of type 2 deiodinase gene in the mediobasal hypothalamus of the Saanen goat, a short-day breeder: implication for seasonal window of thyroid hormone action on reproductive neuroendocrine axis. *Endocrinology* (2006) **147**:432–40. doi:10.1210/en.2005-0507
37. Hanon EA, Lincoln GA, Fustin JM, Dardente H, Masson-Pévet M, Morgan PJ, et al. Ancestral TSH mechanism signals summer in a photoperiodic mammal. *Curr Biol* (2008) **18**:1147–52. doi:10.1016/j.cub.2008.06.076
38. Anderson GM, Hardy SL, Valent M, Billings HJ, Connors JM, Goodman RL. Evidence that thyroid hormones act in the ventromedial preoptic area and the premammillary region of the brain to allow the termination of the breeding season in the ewe. *Endocrinology* (2003) **144**:2892–901. doi:10.1210/en.2003-0322
39. Billings HJ, Viguié C, Karsch FJ, Goodman RL, Connors JM, Anderson GM. Temporal requirements of thyroid hormones for seasonal changes in luteinizing hormone secretion. *Endocrinology* (2002) **143**:2618–25. doi:10.1210/en.143.7.2618
40. Ikegami K, Yoshimura T. Circadian clocks and the measurement of daylength in seasonal reproduction. *Mol Cell Endocrinol* (2012) **349**:76–81. doi:10.1016/j.mce.2011.06.040
41. Abe T, Suzuki T, Unno M, Tokui T, Ito S. Thyroid hormone transporters: recent advances. *Trends Endocrinol Metab* (2002) **13**:215–20. doi:10.1016/S1043-2760(02)00599-4
42. Hagenbuch B, Meier PJ. Organic anion transporting polypeptides of the OATP/SLC21 family: phylogenetic classification as OATP/SLCO superfamily, new nomenclature and molecular/functional properties. *Pflugers Arch* (2004) **447**:653–65. doi:10.1007/s00424-003-1168-y
43. Nakao N, Takagi T, Iigo M, Tsukamoto T, Yasuo S, Masuda T, et al. Possible involvement of organic anion transporting polypeptide 1c1 in the photoperiodic response of gonads in birds. *Endocrinology* (2006) **147**:1067–73. doi:10.1210/en.2005-1090
44. Nicholls TJ, Follett BK, Robinson JE. A photoperiodic response in gonadectomized Japanese quail exposed to a single long day. *J Endocrinol* (1983) **97**:121–6. doi:10.1677/joe.0.0970121
45. Nakao N, Ono H, Yamamura T, Anraku T, Takagi T, Higashi K, et al. Thyrotrophin in the pars tuberalis triggers photoperiodic response. *Nature* (2008) **452**:317–22. doi:10.1038/nature06738
46. Benoit J. Le role des yeux dans l'action stimulante de la lumiere sure le developpement testiulaire chez le canard. *C R Soc Biol (Paris)* (1935) **118**:669–71.
47. Oliver J, Bayle JD. Brain photoreceptors for the photoinduced testicular response in birds. *Experientia* (1982) **38**:1020–9. doi:10.1007/BF01955346
48. Max M, McKinnon PJ, Seidenman KJ, Barrett RK, Applebury ML, Takahashi JS, et al. Pineal opsin: a nonvisual opsin expressed in chick pineal. *Science* (1995) **267**:1502–6. doi:10.1126/science.7878470
49. Okano T, Yoshizawa T, Fukada Y. Pinopsin is a chicken pineal photoreceptive molecule. *Nature* (1994) **372**:94–7. doi:10.1038/372094a0
50. Siopes TD, Wilson WO. Extraocular modification of photoreception in intact and pinealectomized coturnix. *Poult Sci* (1974) **53**:2035–41. doi:10.3382/ps.0532035
51. Menaker M, Roberts R, Elliott J, Underwood H. Extraretinal light perception in the sparrow. III. The eyes do not participate in photoperiodic photoreception. *Proc Natl Acad Sci USA* (1970) **67**:320–5. doi:10.1073/pnas.67.1.320
52. Silver R, Witkovsky P, Horvath P, Alones V, Barnstable CJ, Lehman MN. Coexpression of opsin- and VIP-like-immunoreactivity in CSF-contacting neurons of the avian brain. *Cell Tissue Res* (1988) **253**:189–98. doi:10.1007/BF00221754
53. Wada Y, Okano T, Adachi A, Ebihara S, Fukada Y. Identification of rhodopsin in the pigeon deep brain. *FEBS Lett* (1998) **424**:53–6. doi:10.1016/S0014-5793(98)00138-0
54. Bailey MJ, Cassone VM. Melanopsin expression in the chick retina and pineal gland. *Brain Res Mol Brain Res* (2005) **134**:345–8. doi:10.1016/j.molbrainres.2004.11.003
55. Chaurasia SS, Rollag MD, Jiang G, Hayes WP, Haque R, Natesan A, et al. Molecular cloning, localization and circadian expression of chicken melanopsin (Opn4): differential regulation of expression in pineal and retinal cell types. *J Neurochem* (2005) **92**:158–70. doi:10.1111/j.1471-4159.2004.02874.x
56. Kang SW, Leclerc B, Kosonsiriluk S, Mauro LJ, Iwasawa A, El Halawani ME. Melanopsin expression in dopamine-melatonin neurons of the premammillary nucleus of the hypothalamus and seasonal reproduction in birds. *Neuroscience* (2010) **170**:200–13. doi:10.1016/j.neuroscience.2010.06.082
57. Tomonari S, Takagi A, Akamatsu S, Noji S, Ohuchi H. A non-canonical photopigment, melanopsin, is expressed in the differentiating ganglion, horizontal, and bipolar cells of the chicken retina. *Dev Dyn* (2005) **234**:783–90. doi:10.1002/dvdy.20600
58. Tomonari S, Takagi A, Noji S, Ohuchi H. Expression pattern of the melanopsin-like (cOpn4m) and VA opsin-like genes in the developing chicken retina and neural tissues. *Gene Expr Patterns* (2007) **7**:746–53. doi:10.1016/j.modgep.2007.06.001
59. Davies WI, Turton M, Peirson SN, Follett BK, Halford S, Garcia-Fernandez JM, et al. Vertebrate ancient opsin photopigment spectra and the avian photoperiodic response. *Biol Lett* (2012) **8**:291–4. doi:10.1098/rsbl.2011.0864
60. Halford S, Pires SS, Turton M, Zheng L, Gonzalez-Menendez I, Davies WL, et al. VA opsin-based photoreceptors in the hypothalamus of birds. *Curr Biol* (2009) **19**:1396–402. doi:10.1016/j.cub.2009.06.066
61. Nakane Y, Ikegami K, Ono H, Yamamoto N, Yoshida S, Hirunagi K, et al. A mammalian neural tissue opsin (Opsin 5) is a deep brain photoreceptor in birds. *Proc Natl Acad Sci USA* (2010) **107**:15264–8. doi:10.1073/pnas.1006393107
62. Yamashita T, Ohuchi H, Tomonari S, Ikeda K, Sakai K, Shichida Y. Opn5 is a UV-sensitive bistable pigment that couples with Gi subtype of G protein. *Proc Natl Acad Sci USA* (2010) **107**:22084–9. doi:10.1073/pnas.1012498107
63. Groos GA, van der Kooy D. Functional absence of brain photoreceptors mediating entrainment of circadian rhythms in the adult rat. *Experientia* (1981) **37**:71–2. doi:10.1007/BF01965576
64. Legan SJ, Karsch FJ. Importance of retinal photoreceptors to the photoperiodic control of seasonal breeding in the ewe. *Biol Reprod* (1983) **29**:316–25. doi:10.1095/biolreprod29.2.316
65. Lockley SW, Skene DJ, Thapan K, English J, Ribeiro D, Haimov I, et al. Extraocular light exposure does not suppress plasma melatonin in humans. *J Clin Endocrinol Metab* (1998) **83**:3369–72. doi:10.1210/jc.83.9.3369
66. Meijer JH, Thio B, Albus H, Schaap J, Ruijs ACJ. Functional absence of extraocular photoreception in hamster circadian rhythms entrainment. *Brain Res* (1999) **831**:337–9. doi:10.1016/S0006-8993(99)01509-7
67. Nelson RJ, Zucker I. Absence of extraocular photoreception in diurnal and nocturnal rodents exposed to direct sunlight. *Comp Biochem Physiol* (1981) **69A**:145–8. doi:10.1016/0300-9629(81)90651-4
68. Reiter RJ. The pineal and its hormones in the control of reproduction in mammals. *Endocr Rev* (1980) **1**:109–31. doi:10.1210/edrv-1-2-109
69. Yamazaki S, Goto M, Menaker M. No evidence for extraocular photoreceptors in the circadian system of the Syrian hamster. *J Biol Rhythms* (1999) **14**:197–201. doi:10.1177/074873099129000605
70. Inouye ST, Kawamura H. Persistence of circadian rhythmicity in a mammalian hypothalamic "island" containing the suprachiasmatic nucleus. *Proc Natl Acad Sci USA* (1979) **76**:5962–6. doi:10.1073/pnas.76.11.5962
71. Klein DC, Moore RY, Reppert SM. *Suprachiasmatic Nucleus: The Mind's Clock*. New York: Oxford University Press (1991).
72. Lehman MN, Silver R, Gradstone WR, Kahn RM, Gibson M, Bittman EL. Circadian rhythmicity restored by neural transplant. Immunocytochemical characterization of the graft and its integration with the host brain. *J Neurosci* (1987) **7**:1626–38.
73. Ralph MR, Foster RG, Davis FC, Menaker M. Transplanted suprachiasmatic nucleus determines circadian period. *Science* (1990) **247**:975–8. doi:10.1126/science.2305266
74. Arendt J. *Melatonin and the Mammalian Pineal Gland*. London: Chapman & Hall (1995).
75. Hoffman RA, Reiter RJ. Pineal gland: influence on gonads of male hamsters. *Science* (1965) **148**:1609–11. doi:10.1126/science.148.3677.1609
76. Reppert SM, Weaver DR, Ebisawa T. Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. *Neuron* (1994) **13**:1177–85. doi:10.1016/0896-6273(94)90055-8
77. Reppert SM, Godson CG, Mahle CD, Weaver DR, Slaugenhaupt SA, Gusella JF. Molecular characterization of a second melatonin receptor expressed in human retina and brain: the Mel1b-melatonin receptor. *Proc Natl Acad Sci USA* (1995) **92**:8734–8. doi:10.1073/pnas.92.19.8734

78. Schuster C, Gauer F, Guerrero H, Lakhdar-Ghazal N, Pévet P, Masson-Pévet M. Photic regulation of mt1 melatonin receptors in the Siberian hamster pars tuberalis and suprachiasmatic nuclei: involvement of the circadian clock and intergeniculate leaflet. *J Neuroendocrinol* (2000) **12**:207–16. doi:10.1046/j.1365-2826.2000.00039.x
79. Song CK, Bartness TJ. CNS sympathetic outflow neurons to white fat that express MEL receptors may mediate seasonal adiposity. *Am J Physiol Regul Integr Comp Physiol* (2001) **281**:R666–72.
80. Klosen P, Bienvenu C, Demarteau O, Dardente H, Guerrero H, Pévet P, et al. The mt1 melatonin receptor and RORb receptor are co-localized in specific TSH-immunoreactive cells in the pars tuberalis of the rat pituitary. *J Histochem Cytochem* (2002) **50**:1647–57. doi:10.1177/002215540205001209
81. Wittkowski W, Bergmann M, Hoffmann K, Pera F. Photoperiod-dependent changes in TSH-like immunoreactivity of cells in the hypophysial pars tuberalis of the Djungarian hamster, *Phodopus sungorus*. *Cell Tissue Res* (1988) **251**:183–7. doi:10.1007/BF00215463
82. Ebihara S, Marks T, Hudson DJ, Menaker M. Genetic control of melatonin synthesis in the pineal gland of the mouse. *Science* (1986) **231**:491–3. doi:10.1126/science.3941912
83. Goto M, Oshima I, Tomita T, Ebihara S. Melatonin content of the pineal gland in different mouse strains. *J Pineal Res* (1989) **7**:195–204. doi:10.1111/j.1600-079X.1989.tb00667.x
84. Yasuo S, Yoshimura T, Ebihara S, Kolf HW. Melatonin transmits photoperiodic signals through the MT1 melatonin receptor. *J Neurosci* (2009) **29**:2885–9. doi:10.1523/JNEUROSCI.0145-09.2009
85. Yamamura T, Hirunagi K, Ebihara S, Yoshimura T. Seasonal morphological changes in the neuro-glial interaction between gonadotropin-releasing hormone nerve terminals and glial endfeet in Japanese quail. *Endocrinology* (2004) **145**:4264–7. doi:10.1210/en.2004-0366
86. Prevot V, Croix D, Bouret S, Dutoit S, Tramu G, Stefano GB, et al. Definitive evidence for the existence of morphological plasticity in the external zone of the median eminence during the rat estrous cycle: implication of neuro-glio-endothelial interactions in gonadotropin-releasing hormone release. *Neuroscience* (1999) **94**:809–19. doi:10.1016/S0306-4522(99)00383-8
87. Yamamura T, Yasuo S, Hirunagi K, Ebihara S, Yoshimura T. T_3 implantation mimics photoperiodically reduced encasement of nerve terminals by glial processes in the median eminence of Japanese quail. *Cell Tissue Res* (2006) **324**:175–9. doi:10.1007/s00441-005-0126-8
88. Jansen HT, Cutter C, Hardy S, Lehman MN, Goodman RL. Seasonal plasticity within the gonadotropin-releasing hormone (GnRH) system of the ewe: changes in identified GnRH inputs and glial association. *Endocrinology* (2003) **144**:3663–76. doi:10.1210/en.2002-0188
89. Yoshimura T. Molecular bases for seasonal reproduction in birds. *J Poult Sci* (2004) **41**:251–8. doi:10.1016/j.yfrne.2013.10.002
90. Kasahara T, Abe K, Mekada K, Yoshiki A, Kato T. Genetic variation of melatonin productivity in laboratory mice under domestication. *Proc Natl Acad Sci USA* (2010) **107**:6412–7. doi:10.1073/pnas.0914399107
91. Shimomura K, Lowrey PL, Vitaterna MH, Buhr ED, Kumar V, Hanna P, et al. Genetic suppression of the circadian clock mutation by the melatonin biosynthesis pathway. *Proc Natl Acad Sci USA* (2010) **107**:8399–403. doi:10.1073/pnas.1004368107
92. Rubin CJ, Zody MC, Eriksson J, Meadows JR, Sherwood E, Webster MT, et al. Whole-genome resequencing reveals loci under selection during chicken domestication. *Nature* (2010) **464**:587–91. doi:10.1038/nature08832
93. Nakane Y, Ikegami K, Iigo M, Ono H, Takeda K, Takahashi D, et al. The saccus vasculosus of fish is a sensor of seasonal changes in day length. *Nat Commun* (2013) **4**:2108. doi:10.1038/ncomms3108

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Thyroid hormone upregulates hypothalamic *kiss2* gene in the male Nile tilapia, *Oreochromis niloticus*

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Kisspeptin has recently been recognized as a critical regulator of reproductive function in vertebrates. During the sexual development, kisspeptin neurons receive sex steroids feedback to trigger gonadotropin-releasing hormone (GnRH) neurons. In teleosts, a positive correlation has been found between the thyroid status and the reproductive status. However, the role of thyroid hormone in the regulation of kisspeptin system remains unknown. We cloned and characterized a gene encoding kisspeptin (*kiss2*) in a cichlid fish, the Nile tilapia (*Oreochromis niloticus*). Expression of *kiss2* mRNA in the brain was analyzed by *in situ* hybridization. The effect of thyroid hormone (triiodothyronine, T_3) and hypothyroidism with methimazole (MMI) on *kiss2* and the three GnRH types (*gnrh1*, *gnrh2*, and *gnrh3*) mRNA expression was analyzed by real-time PCR. Expression of thyroid hormone receptor mRNAs were analyzed in laser-captured kisspeptin and GnRH neurons by RT-PCR. The *kiss2* mRNA expressing cells were seen in the nucleus of the lateral recess in the hypothalamus. Intraperitoneal administration of T_3 (5 μg/g body weight) to sexually mature male tilapia significantly increased *kiss2* and *gnrh1* mRNA levels at 24 h post injection ($P < 0.001$), while the treatment with an anti-thyroid, MMI (100 ppm for 6 days) significantly reduced *kiss2* and *gnrh1* mRNA levels ($P < 0.05$). *gnrh2*, *gnrh3*, and thyrotropin-releasing hormone mRNA levels were insensitive to the thyroid hormone manipulations. Furthermore, RT-PCR showed expression of thyroid hormone receptor mRNAs in laser-captured GnRH neurons but not in *kiss2* neurons. This study shows that GnRH1 may be directly regulated through thyroid hormone, while the regulation of Kiss2 by T_3 is more likely to be indirect.

Keywords: cichlid, *in situ* hybridization, hypothalamus, thyroid receptor, kisspeptin

INTRODUCTION

Kisspeptin, encoded by *Kiss1*/*KISS1* (rodents/human) gene and its cognate receptor, GPR54 (=Kiss-R), have recently been considered the major regulator of reproductive functions, in particular the onset of puberty (1). Administration of kisspeptin stimulates gonadotropin secretion (2), either by its direct action on gonadotrophs (3) or through gonadotropin-releasing hormone (GnRH) neurons (4). Variants of *kiss1* homologous sequences (*kiss1* and *kiss2*) have been identified in several non-mammalian vertebrates including amphibian and teleosts (5, 6). In the teleosts brain, cells expressing *kiss1* mRNA are seen in the ventral habenula and/or the ventral hypothalamus, while those of *kiss2* mRNA are seen in the hypothalamic nuclei and/or the preoptic area depending on the fish species (5, 7). With multiple kisspeptin types, multiple forms of Kiss-R encoding genes (*kissr1* and *kissr2*) have been cloned and characterized in various teleosts (5, 6). Several lines of evidence have demonstrated that Kiss2 is more potent than Kiss1 in the control of reproduction in teleosts (8–11). In the sexually mature zebrafish, *Danio rerio*, administration of Kiss2 peptides significantly increases the gonadotropins β-subunit mRNA levels in the pituitary (9). Similarly in prepubertal European sea bass, *Dicentrarchus labrax*, Kiss2 but not Kiss1 injection increases plasma gonadotropins levels (8). We have previously identified Kiss-R (*kissr2*) in the Nile tilapia, *Oreochromis niloticus* and have shown its expression in GnRH neurons (12). These results suggest the potent role of Kiss2 in the reproductive axis during prepubertal development and sexually mature stages in teleosts. In mammals, kisspeptin neurons transmit gonadal steroid feedback signals to GnRH neurons, especially the positive feedback effect of ovarian estrogen that causes the preovulatory GnRH/luteinizing hormone (LH) surge in female (13). Although the *kiss2* gene is highly conserved in non-mammalian vertebrates, a potent trigger of Kiss2 neural activity has not been identified in teleosts. In the medaka, *Oryzias latipes*, only the hypothalamic *kiss1* but not *kiss2* neurons show prominent estrogen sensitivity in their kisspeptin gene expression (14). Similarly in the goldfish, *Carassius auratus*, the preoptic but not hypothalamic *kiss2* neurons show clear estrogen sensitivity (15). In addition, these estrogen sensitive kisspeptin neuron types in the fish express estrogen receptors (ERα and ERβ) (14, 15). In the juvenile zebrafish, *kiss2* neurons are upregulated by estrogen treatment (16). These observations suggest that the hypothalamic Kiss2 neurons can be regulated by ovarian estrogen in a reproductive stage-dependent manner. However, the concept of an estrogen positive feedback mechanism that initiate the

preovulatory GnRH/LH surge is not relevant for males (17). In male aromatase knockout mice, Kiss1 expression in the hypothalamus is not reduced (18). Thus, it is possible that factors other than estrogen play an important role in the regulation of kisspeptin neurons in males (17).

Thyroid hormone is an important regulator of somatic growth, metabolism, brain development, and other vital processes in developing and adult animals (19). Additionally, thyroid hormone also plays an important role in reproductive functions during several physiological conditions (19). In fish, there are numerous studies that examined the effect of hyper- and hypo-thyroidism in sexual development, maturation, and reproductive behavior (20). Direct action of thyroid hormone on GnRH neurons as well as co-expression of thyroid hormone receptors in GnRH neurons has been previously demonstrated (21, 22). In ewe, thyroid hormones are necessary for GnRH and LH pulsatility (23, 24). Although pulsatile secretion of GnRH and kisspeptin are closely interlinked (25), the potential role of thyroid hormone in the regulation of kisspeptin system has never been studied.

In the present study, we cloned *kiss2* cDNA in the Nile tilapia. Gene expression of *kiss2* mRNA in the brain was examined by *in situ* hybridization. Furthermore, to examine the potential role of thyroid hormone in the regulation of the kisspeptin system, the effect of thyroid hormone (triiodothyronine, T_3) and methimazole (MMI) on *kiss2* and GnRH types (*gnrh1*, *gnrh2*, and *gnrh3*) mRNA expression was analyzed by real-time PCR. MMI inhibits thyroperoxidase, which acts in thyroid hormone synthesis by oxidizing the anion iodide (I^-) to iodine (I0), facilitating iodine's addition to tyrosine residues on the hormone precursor thyroglobulin, a necessary step in the synthesis of T_3 and thyroxine (T_4). MMI has been shown to reduce plasma thyroid hormone levels and type III deiodinase (D3) activities (hypothyroid condition) in the brain, gill, and liver of tilapia (26). In the present study, to manipulate the plasma thyroid hormone levels in the male tilapia, we applied two different administration methodologies: for hyperthyroid condition, 24 h after intraperitoneal injection of thyroid hormone while for hypothyroid status, fish were immersed in water containing MMI for 6-days. We also measured mRNA expression levels of thyrotropin-releasing hormone (TRH) to validate the effect of thyroid hormone manipulation. Finally, to confirm the potential mechanism of thyroid hormone action on Kiss2 and GnRH neurons, the expression of thyroid hormone receptor (TR) types (*tra1*, *tra2*, and *trb*) mRNA were analyzed in laser-captured *kiss2* and GnRH neurons.

MATERIALS AND METHODS

ANIMALS

Sexually mature male Nile tilapia, *O. niloticus* (standard length: 11.6 ± 0.4 cm, body weight: 52.6 ± 5.0 g) were maintained in freshwater aquaria at 28 ± 0.5°C with a controlled natural photoregimen (14/10 h, light/dark). They were fed twice daily with commercial tilapia diets (Zeigler, USA). The fish were maintained and used in accordance with the Guidelines of the Animal Ethics Committee of Monash University (Approval Number: SOBSB/2009/58) and Sun Yat-Sen University.

MOLECULAR CLONING OF kiss2 IN THE TILAPIA

The fish were anesthetized by immersing in a 0.01% solution of tricaine methanesulfonate (MS222; Sigma, St. Louis, MO, USA) and killed by decapitation for sample collection. Total RNA from the tilapia brain ($n = 1$) was prepared using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). One microgram of isolated RNA was used to synthesize the first-strand cDNA using the ReverTra Ace-a first-strand cDNA Synthesis Kit (Toyobo, Osaka, Japan). Partial cDNA fragments were obtained by PCR using degenerate primers or gene-specific primers designed based on the sequences of *kiss* genes of fugu, grouper, medaka, and mackerel (**Table 1**). Full-length cDNA sequences were obtained by 5′ and 3′ rapid amplification of cDNA ends (RACE) kit (Invitrogen). For all PCR reactions in this study, amplifications were performed with an initial denaturation step at 94°C for 3 min, followed by 40 cycles of 94°C for 15 s, 55-58°C for 15 s, and 72°C for 30 s. The reaction was ended by a further extension of 10 min at 72°C. The amplification products were purified using the E.Z.N.A. Gel Extraction Kit (Omega BioTek, GA, USA) and ligated into the pTZ57R/T vector (Fermentas, MD, USA). Three different individual positive clones were picked to confirm the sequence information using an ABI 3700 sequencer (Applied Biosystems, Foster City, CA, USA). Putative signal peptides and cleavage sites were predicted using SignalP 3.0[1]. Multiple sequence alignments of amino acids were performed with ClustalX (1.81) program. Protein phylogenetic analysis was conducted with MEGA4 using the neighbor-joining method.

Chromosomal location of tilapia *kiss2* gene was identified and its gene synteny with *kiss2* genes in other teleosts (zebrafish, medaka, *O. latipes*, and puffer fish, *Takifugu rubripes*) were examined using the Ensemble Genome Browser[2].

The tilapia *kiss2* gene promoter sequence was identified *in silico* using the Ensemble Genome Browser. The 2.0-kb sequence upstream of the untranslated region was considered to be the putative promoter. The putative promoter sequence was analyzed for conserved regulatory elements using online bioinformatic tools (TESS[3]; TFSearch[4]; SignalScan[5]).

TISSUE DISTRIBUTION

To determine the tissue distribution of *kiss2* mRNA in the tilapia, sexually mature male and female fish ($n = 1$ each) were anesthetized by immersing in a 0.01% solution of MS222 and killed by decapitation for sample collection. Tissue samples were collected and snap frozen in liquid nitrogen. Total RNA was isolated from the different brain regions (the olfactory bulb, telencephalon, optic tectum thalamus, hypothalamus, cerebellum, and medulla) and peripheral tissues (the pituitary, liver, spleen, intestine, kidney, gill, heart, muscle, testis, and ovary) with TRIzol. One microgram of total RNA from each sample was digested with deoxyribonuclease I (DNase I) and reverse-transcribed into cDNA using the ReverTra Ace-first-strand cDNA Synthesis Kit. PCR was carried

[1]http://www.cbs.dtu.dk/services/SignalP/
[2]http://asia.ensembl.org/index.html
[3]http://www.cbil.upenn.edu/cgi-bin/tess/tess
[4]http://www.cbrc.jp/research/db/TFSEARCH.html
[5]http://www-bimas.cit.nih.gov/molbio/signal/

Table 1 | Primers for tilapia genes used in present study.

Purpose/ genes	Primer direction	5′ to 3′ sequences
5′RACE	Antisense1	AGGCACCTCCAGTTCTCG
	Antisense2	AGCCATTGTAGCGTTTCC
	Antisense3	CTGCCTCTGGTCCTCGTT
	Antisense4	AGTCGCCTGCTGTTCTCC
3′RACE	Sense1	GAACGAGGACCAGAGGCA
	Sense2	TCTCAGCCTTCGCTTTGG
	Sense3	GGGAAACGCTACAATGGC
	Sense4	CTTGCGAGAACTGGAGGTG
ORF	Sense	TTTGGATCTGGTGCTGAA
	Antisense	GTTTGACTTTCCAAACAAAT
TISSUE DISTRIBUTION		
kiss2	Sense	GCTTTGGCTGTGGTTTGC
	Antisense	GCCTCTGGTCCTCGTTCT
β-*actin*	Sense	ATGCCTGGCTGGTCCCTCTGTTCT
	Antisense	GGCGGCCAGGTTTGCTATGTA
REAL-TIME PCR		
kiss2	Sense	TGCACAGAGAACACATGCAA
	Antisense	CTCGCAAGAACAGAGAGAAGG
gnrh1	Sense	CTCGCAGGGACGGTGTTT
	Antisense	TCTTCCCTCCTGGGCTCAGT
gnrh2	Sense	TGGTCCCATGGTTGGTATCC
	Antisense	CCCTGCTTCACACAGCTTAATCT
gnrh3	Sense	TGCTGGCGTTGGTGGTT
	Antisense	CCTCAAGCTCTCCCACACTTCT
trh	Sense	GCAGGATGAAGACGGAAGAAAT
	Antisense	GCCGCTTCTCCAAATCATCA
β-*actin*	Sense	CCTGACAGAGCGTGGCTACTC
	Antisense	TCTCTTTGATGTCACGCACGAT
LOCALIZATION OF TRs		
tra1	Sense	AGTGGAAGCAGAAGCGCAA
	Antisense	TGATGTTGGAGCGACTGGAG
tra2	Sense	CCCATCGTCACACCAATGC
	Antisense	TCACAAGGCAGCAGGAATTTG
trb	Sense	GAAATTCCTGAGTGCAGCGG
	Antisense	CAGGTGCATTACCCGTGGA
β-*actin*		Same as those used for real-time PCR
kiss2		Same as those used for real-time PCR

Primer pairs used for real-time PCR and TRs localization were designed to originate in different exons to exclude false positive bands in case of potential genomic DNA contamination.

out as described above. The PCR products were electrophoresed on 2% agarose gels, stained with ethidium bromide, and visualized by illumination under UV light. All PCR products were confirmed by sequencing.

IN SITU HYBRIDIZATION

Brains of sexually mature males ($n = 3$) were dissected and fixed in buffered 4% paraformaldehyde for 16 h at 4°C. The brains were then cryoprotected in 20% sucrose and embedded in OCT compound (Sakura Finetechnical, Tokyo, Japan). Consecutive coronal sections (15 μm thick) were cut on a cryostat and thaw-mounted onto 3-aminopropylsilane-coated glass slides. Sense and antisense digoxigenin (DIG)-labeled riboprobes were synthesized from partial sequence of tilapia *kiss2* (266 nt) using MAXIscript (Ambion, Austin, TX, USA) and DIG RNA Labeling Mix (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instruction. DIG-*in situ* hybridization was performed as described previously (9). Briefly, sections were subjected to permeabilization with 0.2M HCl for 10 min followed by proteinase K (1 μg/ml) treatment for 15 min, and hybridized with the DIG-labeled riboprobes (50 ng/ml) at 58°C overnight in a humidified chamber. After hybridization, sections were washed and blocked with 2% normal sheep serum. DIG signals were detected with an alkaline phosphatase-conjugated anti-DIG antibody (Roche Diagnostics, diluted 1:500) with 4-nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate (Roche Diagnostics).

THYROID HORMONE TREATMENT AND INDUCTION OF HYPOTHYROIDISM

To induce hyperthyroidism, thyroid hormone (T_3) was administered as described previously (22). Briefly, anesthetized sexually mature male fish were intraperitoneally (IP) injected with 30 μl of T_3 (3,3′,5-Triiodo-L-thyronine sodium salt, Sigma, US; dissolved in 100% ethanol and then diluted with sesame oil) at 5 μg/g BW or sesame oil (control) through a 25-gage syringe needle ($n = 15$/group, single injection). The selected dose of T_3 has been reported to produce plasma T_3 levels of 4.6 ± 1.2 ng/ml in the male tilapia 24 h after the injection (22), which is within the physiological levels ($2 \sim 5$ ng/ml) in the Mozambique tilapia, *O. mossambicus* (27). After the injection, the fish were released into the recovery tank. Twenty-four hours after the injection, the fish were anesthetized and killed by decapitation, and the brain was dissected for RNA isolation.

To induce hypothyroidism, fish were treated in water containing 100 ppm of methimazole (2-Mercapto-1-methylimidazole, MMI, Sigma; dissolved in 100% ethanol and then diluted in water) or in water containing equal volume of 100% ethanol ($n = 5 \sim 8$/group) for 6-days. The water containing MMI was changed every day, which was required to reduce amount of endogenous T_4 levels from a euthyroid to hypothyroid state similar to the treatment in tilapia treated with MMI (26). The selected dose of MMI was calculated based on the concentrations that have been applied to the Nile tilapia and the sea bream, *Sparus auratus*, via diet in previous studies (26, 28). After the treatment, the brain tissue was dissected and frozen on dry ice, and stored at −80°C until use for RNA isolation.

REAL-TIME PCR FOR *kiss2*, *gnrh1*, *gnrh2*, *gnrh3*, AND *trh* GENES

Total RNA was extracted from the brain with TRIzol (Invitrogen) and 200 ng of total RNA were subjected to cDNA synthesis using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) in a final volume of 20 μl reaction mixture containing 1× RT buffer, 1× dNTP mix, 1× RT Random Primers, 20U ribonuclease inhibitor, and 10U MultiScribe Reverse Transcriptase according to the manufacturer's instruction. The cDNA samples were then subjected to real-time PCR for tilapia *kiss2*, *gnrh1*, *gnrh2*, *gnrh3* (GenBank accession numbers for three GnRH types: AB104861, AB101666, and AB104863) and β-actin (*b-actin*)

mRNAs with an ABI PRISM 7500 Sequence Detection System (Applied Biosystems). In addition, effect of thyroid hormone manipulation on TRH (also known as thyroliberin: GenBank Accession number, XM_003438996) was also examined. The PCR reaction mixture (10 μl) contained 1× POWER SYBR Green PCR Master Mix (Applied Biosystems), 0.1 μM each forward and reverse primer, and 1 μl of sample cDNA. Nucleotide sequences of real-time PCR primers for tilapia *kiss2*, GnRH types, *trh* and β-actin are presented in **Table 1**. Reactions were carried out at 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 1 min followed by a dissociation stage. The cycle threshold (Ct) values of all genes was determined and normalized against β-actin mRNA levels. Data was then analyzed according to relative gene expression by $2^{-\Delta\Delta Ct}$. To check PCR specificity, representative PCR products were electrophoresed on 2% agarose gels, stained with ethidium bromide, and visualized by illumination under UV light. Nucleotide sequences of the PCR products were further confirmed by sequencing. Data are expressed as mean ± SEM and statistical analysis was performed by one-way ANOVA followed by *post hoc* analysis with *t*-test for parametric data or the Mann–Whitney *U* test for non-parametric data with $P < 0.05$ considered significant.

EXPRESSION OF TR TYPES IN LASER-CAPTURED kiss2 AND GnRH NEURONS

The expression of TR mRNA types (tra1, tra2 and trb) was examined in laser-captured DIG-labeled *kiss2* neurons and immunolabeled GnRH types neurons by RT-PCR. The fish were anesthetized by immersing in a 0.01% solution of MS222 and killed by decapitation for sample collection. Brains of sexually mature male fish were processed for DIG-*in situ* hybridization of *kiss2* gene and immunofluorescence labeling of three GnRH neurons types ($n = 3$ for each cell types). DIG-*in situ* hybridization for *kiss2* was performed as described above. For harvesting of GnRH neurons, the brain sections were stained with rabbit anti-tilapia GnRH antibodies against their respective GnRH associated peptide (GAP) sequence (GAP1, #ISP105; GAP2, #ISP205, and GAP3, #ISP305), which were previously generated in our lab. Dilutions (1:1000) were made in an RNase-free phosphate buffer saline (pH 7.0) containing 2% bovine serum albumin and 0.5% triton X-100, and the antiserum was applied to sections mounted on slides for 24 h in a closed moist chamber at 4°C and detected with Alexa Fluor 546-labeled anti-rabbit IgG (1:500 dilution, Invitrogen, Carlsbad, CA, USA). DIG-labeled *kiss2* and immune-fluorescently labeled GnRH neurons were laser-microdissected using an Arcturus XT system (Molecular Devices, Sunnyvale, CA, USA). Each population of the laser-microdissected cells (*kiss2*, ∼200 cells; GnRH1, ∼100 cells; GnRH2, ∼30 cells; GnRH3, ∼30 cells/fish) were placed into sterile 0.2 ml PCR tubes containing 50 μl of lysis solution [1× RT buffer (Applied Biosystems, Foster City, CA, USA), 1% Nonidet P-40, and 0.05 mg/μl proteinase K] and lysed for 1 h at 50°C. After DNase I treatment, total RNA was isolated using TRIzol (Invitrogen) and dissolved in a 10-μl of DEPC-treated water. The total RNAs were subsequently subjected to cDNA synthesis as above. The cDNA samples were then subjected to RT-PCR for tilapia *tra1*, *tra2*, and *trb* (GenBank accession numbers: AF302248, AF302249, and AF302247), β-actin (*b-actin*) and *kiss2* mRNAs. The PCR mixture (20 μl) contained 1× PCR buffer, 160 μM of dNTP mix, 250 nM of forward and reverse gene-specific primers (**Table 1**), 1U of AmpliTaq Gold DNA polymerase (Applied Biosystems), and one 20th of a single cell's cDNA solution. Reaction conditions for PCR were 94°C for 10 min, 40 cycles at 94°C for 15 s, 60°C for 15 s, 72°C for 15 s, and 72°C for 7 min. PCR products were electrophoresed on 2% agarose gels, stained with ethidium bromide, and visualized by illumination under UV light.

DOUBLE-IMMUNOFLUORESCENCE OF GnRH1 FIBERS OR GnRHR WITH kiss2 NEURONS

To confirm possible associations between GnRH1 and Kiss2 neurons, double-labeling was performed. Kiss2 neurons were detected by fluorescent *in situ* hybridization, while GnRH1 and GnRHR was detected by immunofluorescence. *kiss2* mRNA expressing cells were detected using NEN Fluorescein Tyramide Signal Amplification (TSA™) Plus kit (Perkin Elmer, Wellesley, MA, USA) according to the manufacturer's instruction. GnRH1-immunoreactive fibers were detected with the anti-tilapia GAP1 antibody (#ISP105, dilution of 1:1000) or anti-tilapia GnRHR [#ISPR3, dilution of 1:500; (29)] with Alexa Fluor 594-labeled anti-rabbit IgG (1:500 dilution, Invitrogen). Separate images were captured by using a microscope (ECLIPS 90i, Nikon Instruments) that was attached to a digital cool CCD camera (DMX1200, Nikon) with appropriate excitation for Fluorescein and Alexa Fluor 594, and a computer software (NIS Elements D3.0, Nikon) was used to superimpose the two images. The red channel was then converted to magenta, and brightness and contrast adjustments were made in Adobe Photoshop CS2 (Adobe Systems, San Jose, CA, USA).

RESULTS

CLONING AND SEQUENCE ANALYSIS OF TILAPIA kiss2 cDNA

A full-length cDNA encoding the *kiss2* precursor was isolated from the tilapia, and the cDNA sequence has been deposited in the GenBank (accession number JN565693). The cDNA encoding tilapia *kiss2* is 633 base pairs (bp), containing an open reading frame of 375 bp, 35 bp of 5′-UTR, and 223 bp of 3′-UTR. The Kiss2 precursor protein has 124 amino acids (aa), with an N-terminal putative signal peptide sequence of 23 aa and a cleavage site (GKR) (**Figure 1A**). Sequence comparison of the deduced protein sequences showed that the tilapia and other vertebrate Kiss precursor proteins are poorly conserved (**Figure 1B**). However, the mature peptide (Kiss2–10) of tilapia and other species exhibit relatively conserved, differing by two amino acid at the position 6 and 7 (phenylalanine to leucine and glycine to serine) (**Figure 1B**). Phylogenetic analysis showed that kisspeptin deduced protein sequences are clustered into two separate clades: Kiss1 and Kiss2. The tilapia Kiss2 is clustered with the Kiss2 clade and shares the highest similarity with sea bass and grouper Kiss2 (**Figure 2A**).

GENE SYNTENY ANALYSIS

Tilapia Kiss2 encoding sequence was found in the chromosome, scaffold GL831328.1 (location 1,353,904–1,355,714). Chromosome synteny analysis revealed that the neighborhood genes around the tilapia *kiss2* including *ldhba* and *slc25a3* are conserved in other fish Kiss2 genes (**Figure 2B**). Some of gene loci nearby the tilapia Kiss2 including *goltlba* and *gys2* were also found on

A

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  1 ACATGGGGAGACAAACAATTTTTGGATCTGGTGCTGAAAGATATGAGACTACTGGCTTTG  60
                                                  M  R  L  L  A  L
 61 GCTGTGGTTTGCGCTCTCATTGCTATCCAGGATGGAGGGAGTGTGGGAGCAGCTCTGCCA 120
     A  V  V  C  A  L  I  A  I  Q  D  G  G  S  V  G  A  A  L  P
121 GGAGTCGACCCTGCACAGAGAACACATGCAACAGGAGCAGTGTCCTCTGCATTCAGGAGA 180
      G  V  D  P  A  Q  R  T  H  A  T  G  A  V  S  S  A  F  R  R
181 ACAGCAGGCGACTTCCTGGCAGAGGATCCCAGCCTCTGCTTTTCCCTGAGAGAGAACGAG 240
     T  A  G  D  F  L  A  E  D  P  S  L  C  F  S  L  R  E  N  E
241 GACCAGAGGCAGCTCCTTTGCAATGATCGCAGAAGTAATTTCAACTACAACCCTCTCAGC 300
     D  Q  R  Q  L  L  C  N  D  R  R  S  N  F  N  Y  N  P  L  S
301 CTTCGCTTTGGGAAACGCTACAATGGCTACATTTACAGAAGAGCTGTTAAAAGAGCCAGA 360
    L  R  F  G  K  R  Y  N  G  Y  I  Y  R  R  A  V  K  R  A  R
361 ACAAAAAAGTTTTCACCCTTCTCTCTGTTCTTGCGAGAACTGGAGGTACCCACCTGAAAC 420
     T  K  K  F  S  P  F  S  L  F  L  R  E  L  E  V  P  T  *
421 AGAAGACTTTCTCTGGGGAATTATGTTATTTGTTTGGAAAGTCAAACTGTGACAGCAGTG 480
481 TTCTTAAAACTCTTTATTTCAGAAAAAAAGGTTTCCCTGATTAAAACTTTTGCACCTATC 540
541 TTTAATGTAAAATAATTTTCAGATGCTACAATGGAGAGAACTATGTAAATAAAATACTTT 600
601 TAGAGAGCTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGT 642
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B

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Grouper kiss1    ---MPR-LIVALMMAALST--EVCTTG-SLKSTYHSEDQRVLKALRDLSHASIPPSAKSS
Seabass kiss1    ---MPR-LIVALMIAALST--EIYNT--SMISSYHSKDQVILKALRDLSHASILASAKNS
Medaka kiss1     ---MAAPLIVAVIMWAVLA--QVWTAHHRHQSTIHTEDNALLKMLRNFNYLS--SSMKEW
Zebrafish kiss1  ----MMLLTVILMLSVARV--HTNPSG--HFQYYLEDETPEETSLRVLRGTDTRPTDGSP
Goldfish kiss1   ----MKLLTIILMLSVANG--DPYPSG--HFQYYLEDETPKE-SLQVLRGTDTRPMAGSP
Grouper kiss2    ----MRLVTLVVVCGLIVG--QDGDGVGAALPGFDSAQRTRATGSILSALRRR-------
Seabass kiss2    ----MRLVALVVVCGLILG--QDGGSVGAALPELDSAQRTGATGSLLSALRRR-------
Tilapia kiss2    ----MRLLALAVVCALIAI--QDGGSVGAALPGVDPAQRTHATGAVSSAFRR--------
Medaka kiss2     ----MTRAVVLVLCALIAA--QDGGRAAAGLAARDSGRGTHATG-VLWILRR--------
Fugu kiss2       ----MRVLVLLLVLAVAPD--RGG--------AHATMQVTGGSG--SVQLRRG-------
Zebrafish kiss2  ---MNTRALILFMSAMVSQ--STAMRAILTDMDTP--EPMPDPKPRFLSMERR-------
Goldfish kiss2   ---MKIKALILFMSAMICQ--STALRASFTDMDISDSEPVPDSKQHYLSVERR-------
Lamprey kiss2    MTPACSLAALLAVCVFGGGAVAARTDRYGASPDSNHARRARSSEEIVTGDLRASPLRLFG
Lamprey kiss1    -MRGLTVVTFLFLVLCCDSFGKVVSFYGFKESTKSGGGQLPGDVTDILREITSLLEGTDG

Grouper kiss1    VNL--------------PADRVHSADGKFPRSGWWI-SKVIFPQTIKKHQDVSSYNLNSF
Seabass kiss1    GNL--------------PADKVHSADGKFPRSEWLI-SKLVLPQTIKKRQDVSSYNLNSF
Medaka kiss1     P----------------KSDR--SSDGGTPMVGCWM-VKALHPVAIKKRQDLSSYNLNSF
Zebrafish kiss1  PSK--------------LSALFSMGAGPQKNTWWWS-PESPY---TKRRQNVAYYNLNSF
Goldfish kiss1   SPK--------------LSVHFSMSADPQRNTRWWA-PVRPY---TKRKQNVWYYNLNSF
Grouper kiss2    -----------------STGEF-VAEDTSPCLSLRE-NEEQRQLLCND--RRSKFNFNPF
Seabass kiss2    -----------------TAGEF-FGEDSSPCFSLRE-NEEQRQLLCND--RRSKFNFNPF
Tilapia kiss2    -----------------TAGDF-LAEDPSLCFSLRE-NEDQRQLLCND--RRSNFNYNPL
Medaka kiss2     -----------------SEDDS-AAGGAGLCSSLRE-DDEQ--LLCAD--RRSKFNYNPF
Fugu kiss2       -----------------TAGQLQLLQESNPCLTFRD-NEDQ--LLCN----RSKFNLNPF
Zebrafish kiss2  -----------------QFEEPSASDDASLCFFIQE-KDETSQISCKHRLARSKFNYNPF
Goldfish kiss2   -----------------QFDEPSSSDDASLCFFFQE-KDESTHISCQHRLPRGKFNYNPF
Lamprey kiss2    AVCR----------HAAETPRLLRLRALRGGHDLEAGLTDGEALPRSAEQDVTHFNYNPF
Lamprey kiss1    IVAFYDFP-------GSGGSVDRAFMSPLHFYPMLRARMRSLPASDADEKKGSTYNWNSF

Grouper kiss1    GLRYGK-------------------------------------------
Seabass kiss1    GLRYGK-------------------------------------------
Medaka kiss1     GLRYGK-------------------------------------------
Zebrafish kiss1  GLRYGKREQDMLTRLIQKSPVK----------------------------
Goldfish kiss1   GLRYGKREQNMLAEFKQKIPMK----------------------------
Grouper kiss2    GLRFGKRYNGYIYRRAVKAARTNKFSPFSLFSRELEVPS-----------
Seabass kiss2    GLRFGKRY---IYRRALKRARTNRFSPLFLFSRELEVPT-----------
Tilapia kiss2    SLRFGKRYNGYIYRRAVKRARTKKFSPFSLFLRELEVPT-----------
Medaka kiss2     GLRFGKRAP---PPRGAHRARAMKLPLMSLFQ---EVPT-----------
Fugu kiss2       GLRFGKRF---IYRRAMKQARTHTRSPV-----SQEVPT-----------
Zebrafish kiss2  GLRFGKRNE--ATTSDSDRLKHKHLLPMMLYLRKQLETS-----------
Goldfish kiss2   GLRFGKRNE--APT---DRPKHKHLLPMMIYLRKQSETT-----------
Lamprey kiss2    GLRFGRRSGAQSSTAATRSRAEAACAPGKRGCRLVISKFKLRF-------
Lamprey kiss1    GLRFGKRELNFMNISKILIIFTKRQ-------------------------
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FIGURE 1 | cDNA and deduced amino acid sequence of tilapia *kiss2*. **(A)** Nucleotide and deduced amino acid sequence of tilapia *kiss2*. Putative signal peptide (underlined) were predicted using SignalP 3.0 (http://www.cbs.dtu.dk/services/SignalP/). Putative core peptide is boxed. Potential cleavage amidation site (GKR) is bolded. The stop codon is denoted by an asterisk. **(B)** Comparison of amino acid sequences of kisspeptin precursors from different species. The mature peptides and potential cleavage amidation site (GKR/GK/GKK) are boxed. Sequences were aligned by the ClustalW program. Gaps (indicated by hyphens) are introduced in some sequences to maximize alignment.

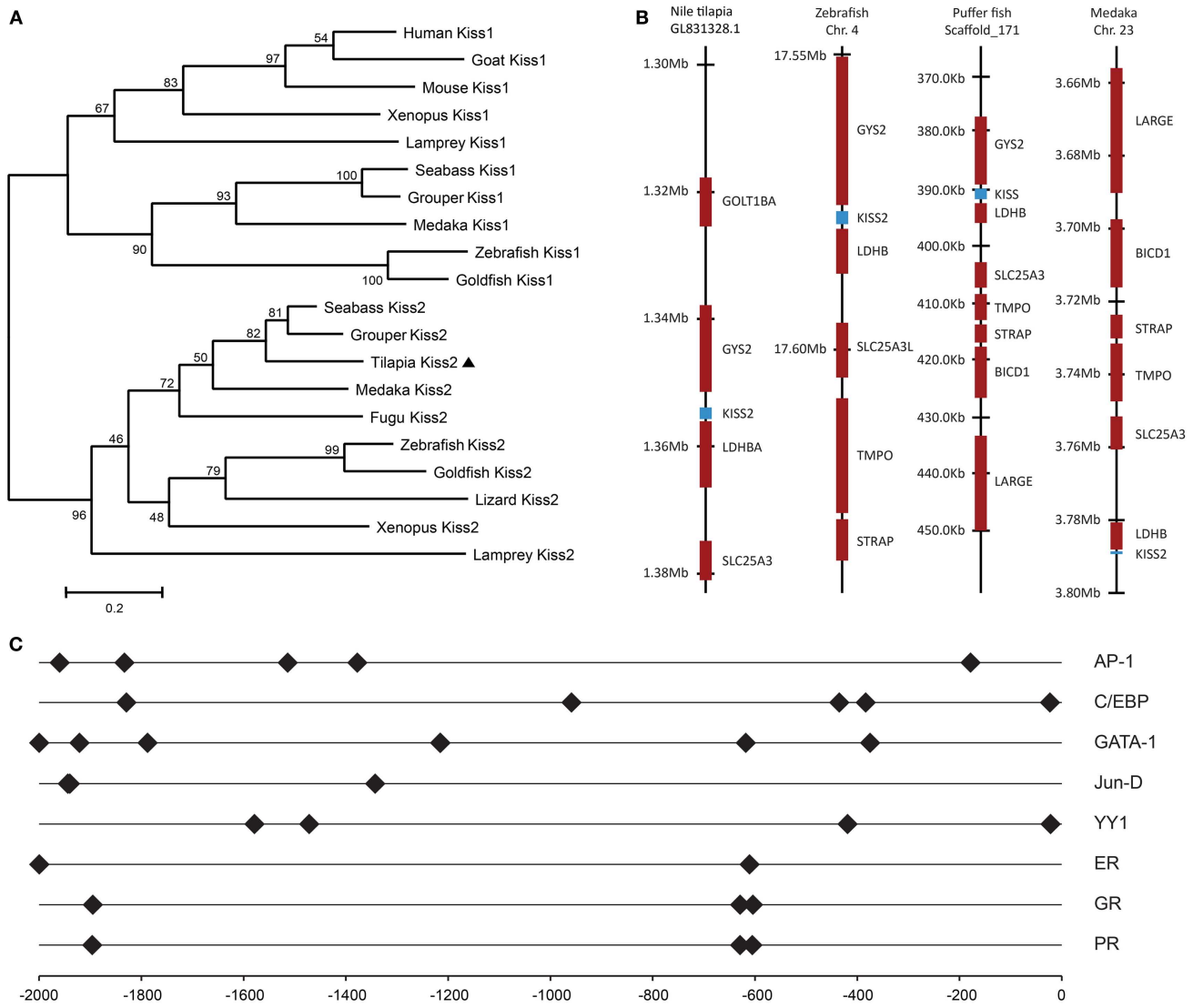

FIGURE 2 | Genomic analysis of tilapia *kiss2* gene. **(A)** Phylogenetic analysis of KISS precursors in vertebrates. The phylogenetic tree was constructed by MEGA 4.0.2 using the neighbor-joining method with 1000 bootstrap replicates. The number shown at each branch indicates the bootstrap value (%). GenBank accession numbers for KISS: human KISS1 (NP_002247.3); Mouse KISS1 (AAI17047.1); Goat KISS1 (ACI96030.1); Clawed frog Kiss1 (NM_001170453.1); Clawed frog Kiss2 (NM_001162860.1); Zebrafish Kiss1 (NP_001106961.1); Zebrafish Kiss2 (NP_001136057.1); Grouper Kiss1 (ADF59544.1); Grouper Kiss2 (ADF59545.1); Medaka Kiss2 (NP_001153913.1); Fugu Kiss2 (BAJ15497.1); Sea bass Kiss1 (ACM07422.1); Sea bass Kiss2 (ACM07423.1). Sequences predicted from Ensembl: sea lamprey Kiss2 (Contig Contig37453.1 at location 1700–6241); Lizard Kiss2 (on scaffold_15 at location 4,601,534–4,601,935); Sea lamprey Kiss1 sequence were previously predicted by van Aerle et al. (30); Goldfish Kiss2 were obtained by Li et al. (31). **(B)** Chromosomal locations *kiss2* (blue box) in various teleosts species. **(C)** Putative transcription factor binding sites (closed diamond) on the promoter region of tilapia *kiss2* gene. The numbers −2000 to 0 represent distance in bp from the putative transcriptional initiation site.

human chromosome 12 and mouse chromosome 6 as reported previously (9).

PUTATIVE TRANSCRIPTION FACTOR BINDING SITES ON THE PROMOTER REGION OF TILAPIA *kiss2* GENE

In silico analysis of putative transcription binding sites showed the presence of binding sites for several transcription factors such as AP-1, CEBP, GATA-1, Jun-D, YY1, ER, GR, and PR on the putative promoter region of tilapia *kiss2* gene (**Figure 2C**).

TISSUE DISTRIBUTIONS AND BRAIN LOCALIZATION OF TILAPIA kiss2 mRNA

RT-PCR analysis was performed to examine the tissue distribution patterns of the tilapia *kiss2* gene expression. In the brain, the tilapia *kiss2* mRNA was highly expressed in the hypothalamus and the pituitary in males and females (**Figure 3**). In peripheral tissues, there were sexual differences in the distribution patterns. In males, the tilapia *kiss2* mRNA was expressed in the spleen, medulla, gills, and testis, whereas in females, the tilapia *kiss2* mRNA was

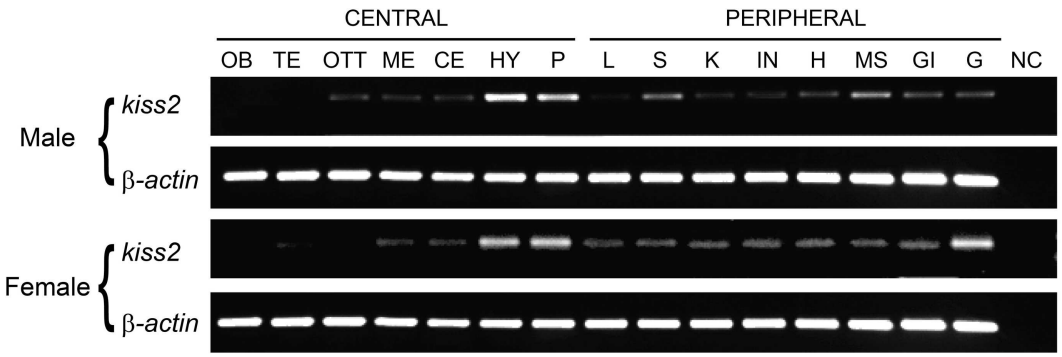

FIGURE 3 | RT-PCR analysis of tissue expression patterns of *kiss2* in male and female tilapia. Amplification of β-actin was used as the house-keeping gene control. OB, olfactory bulb; TE, telencephalon; OTT, optic tectum thalamus; ME, medulla; CE, cerebellum; HY, hypothalamus; P, pituitary; L, liver; S, spleen; K, kidney; IN, intestine; H, heart; MS, muscle; GI, gill; G, gonad; NC, negative control.

expressed in the spleen, kidney, intestine, heart, medulla, gills, and ovary (**Figure 3**).

Digoxigenin-*in situ* hybridization showed tilapia *kiss2* mRNA containing cells in the nucleus of the lateral recess [nRL, also been referred to as the dorsal zone of the periventricular hypothalamus (32)] in the brain (**Figure 4**). No DIG-labeled cells were detected in the brain using sense riboprobes (data not shown).

EFFECT OF THYROID HORMONE (T_3) AND HYPOTHYROIDISM ON kiss2, GnRH TYPES AND TRH GENE EXPRESSION

Real-time PCR showed that administration of T_3 significantly increased the amount of *kiss2* (∼2.3-fold, $P < 0.001$) and *gnrh1* (∼3.2-fold, $P < 0.001$) mRNA levels 24 h post administration when compared with control fish (**Figure 5A**). There was no effect of T_3 treatment on *gnrh2* ($P = 0.86$) and *gnrh3* ($P = 0.47$) mRNA levels (**Figure 5A**).

In the fish treated with MMI, the amount of *kiss2* (∼0.1-fold, $P < 0.05$) and *gnrh1* (∼0.6-fold, $P < 0.05$) mRNA levels were significantly decreased compared with control fish (**Figure 5B**). There was no effect of MMI treatment on *gnrh2* ($P = 0.08$) and *gnrh3* ($P = 0.14$) mRNA levels (**Figure 5B**).

There was no significant effect of thyroid hormone injection and MMI treatment on TRH mRNA levels in the brain (**Figure 6**), indicating the absence of endogenous thyroid hormone feedback effect on *kiss2* mRNA levels.

EXPRESSION OF TR TYPES IN LASER-CAPTURED kiss2 AND GnRH NEURON TYPES

RT-PCR showed no expression of TR types (*tra1*, *tra2*, and *trb*) mRNA in laser-captured *kiss2* cells (**Figure 7**). In GnRH neuron types, expression of *tra1* mRNA was found in GnRH1 and GnRH2 neurons, *tra2* mRNA was found in GnRH3 neurons, and *trb* mRNA was found in GnRH1, GnRH2, and GnRH3 neurons (**Figure 7**).

POSSIBLE NEURONAL ASSOCIATIONS BETWEEN GnRH1 AND kiss2 NEURONS

Double-immunofluorescence showed neither close association of GnRH1-immunoreactive fibers with *kiss2* neurons (**Figures 8A–C**) nor co-expression of GnRHR-immunoreactivity in *kiss2* neurons (**Figures 8D–F**).

DISCUSSION

Kiss2 GENE IN THE TILAPIA

The core sequence of tilapia Kiss2 showed high similarities with non-mammalian Kiss2 peptides sharing the F–F form. However, there are the two major amino acid substitutions at positions 6 and 7 (Leu-Ser instead of Phe-Gly) in the core sequence of tilapia Kiss2 decapeptides. As a result, the carboxyl half of core peptide of tilapia Kiss2 (position 6 to 10) is LSLRF, while those of all other Kiss2 identified thus far are FGLRF with complete conservation from lamprey, elephant shark through platypus that have been appeared to possess Kiss2 (7). These two amino acids substitution could be important for binding affinity to Kiss-R and calcium release activity (33). Comparison of genomic sequences showed conserved synteny between the tilapia, zebrafish, puffer fish and medaka, suggesting tilapia Kiss2 gene is ortholog. So far no *kiss1* and *kissr1* homologous sequences have been reported in the tilapia, similar to those fish that possess only one *kiss-kissr* gene (5, 11). Nevertheless, the presence of *kiss1* in the tilapia remains to be examined.

The expression pattern of *kiss2* mRNA in various tissues in the tilapia is similar to that in other fish species (5, 7). In the brain, *kiss2* mRNA containing cells were seen only in the nRL. However, no *kiss2* cells were seen in other brain region such as the posterior tuberal nucleus or the preoptic area where *kiss2* cells exist in the medaka, zebrafish, goldfish, red seabream (*Pagrus major*) and European sea bass (*D. labrax*) (9, 15, 34–36), which could be due to species difference or because of its low expression levels in the preoptic region. Expression of kisspeptin genes in the pituitary have been reported in several species including mammals and fish (7, 36, 37). The presence of *kiss2* mRNA in the pituitary of tilapia indicates the possibility of *kiss2* mRNA being transported to the nerve terminals as seen in some neuron types (38), or being expressed locally in the pituitary similar to *kiss1* mRNA in the European sea bass and Kiss2-immunoreactive cells in the zebrafish (16, 36). The target site of tilapia Kiss2 neurons is still unknown due to the lack of specific antibody. A recent study in the zebrafish has demonstrated projections

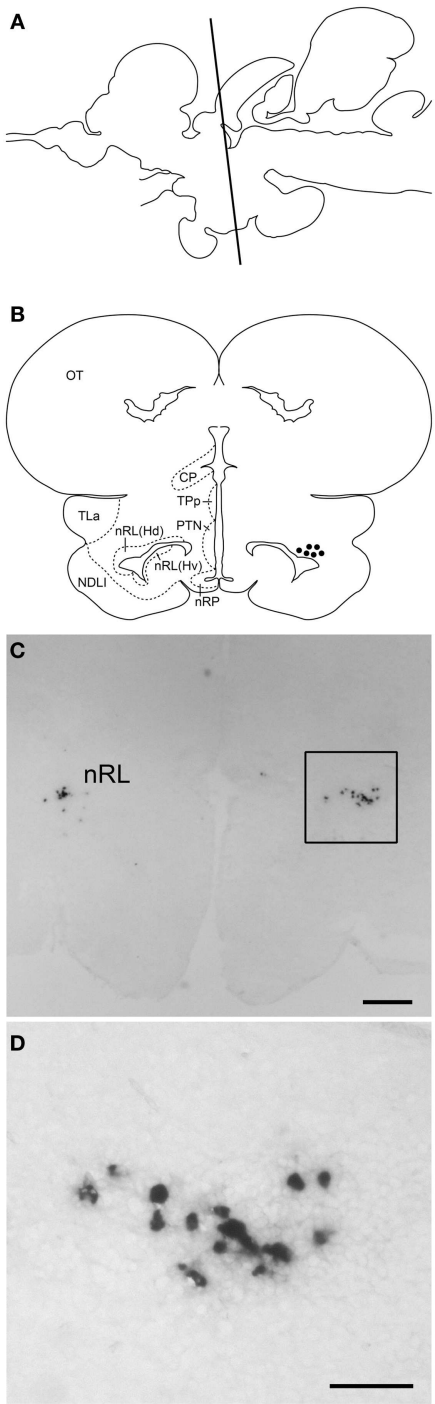

FIGURE 4 | *kiss2* mRNA containing cells in the brain of male tilapia. **(A)** Schematic drawing of a lateral view of the brain indicating levels of the corresponding transverse section. **(B)** Schematic drawing of transverse section of *kiss2* cells (closed circles). **(C,D)** Photomicrographs of DIG-labeled *kiss2* cells in the nucleus of lateral recesses (nRL) at **(C)** Low- and **(D)** high-magnification. CP, central posterior thalamic nucleus; Hd, dorsal zone of periventricular hypothalamus; Hv, ventral zone of periventricular hypothalamus; NDLI, diffuse nucleus of the inferior lobe; nRP, nucleus of posterior recess; OT, optic tectum; PTN, posterior tuberal nucleus; TLa, torus lateralis; TPp, periventricular nucleus of posterior tuberculum. Scale bars: **(C)** 200 μm; **(D)** 50 μm.

of Kiss2-immunoreactive fibers throughout the brain and their close association with GnRH3 (hypophysiotropic GnRH type in the zebrafish) neurons in the preoptic area (16), which suggest the primary role of Kiss2 neurons in gonadotropin secretion possibly through the stimulation of GnRH. It has been shown that electrical stimulation of the nRL in teleosts elicits feeding, gravel picking, and generally aggressive behaviors in cichlids (39). A recent study has shown significant increase in *kiss2* mRNA levels in the hypothalamus during fasting conditions in the Senegalese sole (*Solea senegalensis*) (40). These observations suggest the potential role of Kiss2 in homeostatic regulation as well as ingestive and sexual behaviors as suggested in mammals (41).

The predominant expression of *kiss2* in the brain, testis, and ovary suggests its role in reproductive functions. Specific localization of *kiss2* mRNA in the gonadal tissues has not been studied in teleost, but in the cyclic human and marmoset ovaries, kisspeptin-immunoreactive signals have been located in the theca layer of growing follicles, corpora lutea, interstitial gland, and ovarian surface epithelium (42). Similarly in teleosts, Kiss2 peptides could be locally synthesized in gonadal tissues and could regulate gonadal maturation.

EFFECT OF THYROID HORMONE ON REPRODUCTIVE NEUROENDOCRINE SYSTEM

The manipulation of thyroid hormone levels significantly altered *kiss2* mRNA levels along with *gnrh1* mRNA levels in the brain of male tilapia. Furthermore, there was no effect of thyroid hormone manipulation on *gnrh2* and *gnrh3* mRNA levels. These results indicate that thyroid hormone may act on kisspeptin-GnRH1 system which plays an important role in the reproductive neuroendocrine axis in the tilapia. A recent study in primates has proposed kisspeptin neurons as candidate action target of thyroid hormone (43). The regulation of GnRH neurons by kisspeptin is critical for the onset of puberty. During the prepubertal stage, sex steroids as well as thyroid hormone are involved in the development of the sexually mature brain. In the quail, thyroid hormone has been reported to cause seasonal change in the morphology of GnRH nerve terminals at the median eminence (44). In monkeys, hypothyroid condition with MMI treatment during the juvenile stage delays the pubertal rise in LH secretion and only 50% of the hypothyroid animals exhibit reactivation of GnRH pulse generator activity (45). In teleosts, there are limited studies that examined the role of thyroid hormone in the regulation of GnRH neurons. In the larval tilapia, the concentration of thyroid hormone levels in the whole-body peak around day 25 after hatching (46), which correspond with the period when GnRH1-immunoreactive cells are morphologically detectable in the preoptic area (47). In the zebrafish, the timing of first appearance of preoptic GnRH3 neurons and that of the increase in *gnrh3* gene expression coincides with the second peak of *kiss2* gene expression (9). However, these studies only support the potential organizational effect of thyroid hormone on the reproductive axis in juvenile or seasonal breeding animals, which is not the case for the present study that demonstrates the activational effect of thyroid hormone on kisspeptin-GnRH axis in the sexually mature fish. Nevertheless, even in non-seasonal breeding animals, thyroid hormone levels are influenced by various factors such as diurnal rhythm

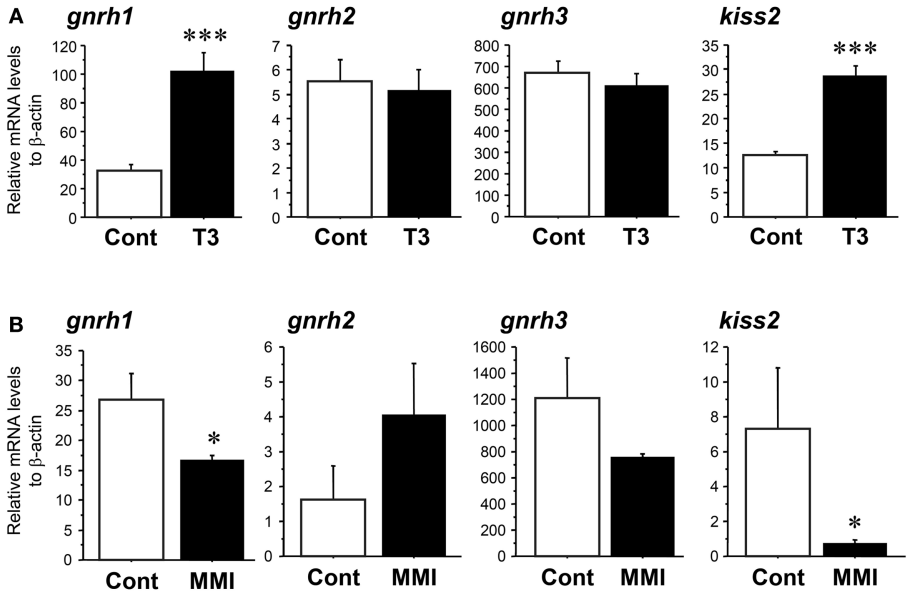

FIGURE 5 | Effect of thyroid hormone manipulation in *kiss2, gnrh1, gnrh2,* and *gnrh3* mRNA levels in the male tilapia. **(A)** Thyroid hormone (T_3, 5 μg/g body weight) injection significantly increased *kiss2* and *gnrh1* mRNA levels ($n = 15$). **(B)** Under hypothyroidism with methimazole (MMI, 100 ppm for 6 days), mRNA levels of *kiss2* and *gnrh1* were significantly decreased. There were no effects of manipulation of thyroid hormone on *gnrh2* and *gnrh3* mRNA levels ($n = 5$–8). The relative abundances of the mRNA were normalized to the amount of β-actin using the comparative threshold cycle method. $^*P < 0.05$; $^{***}P < 0.001$ vs. controls.

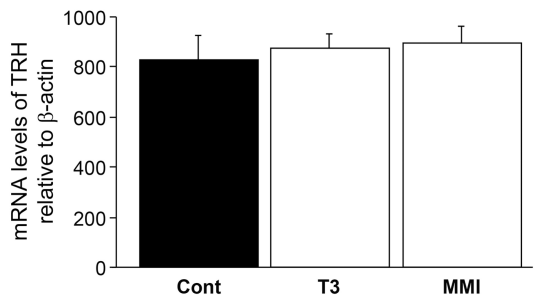

FIGURE 6 | Effect of thyroid hormone manipulation on thyrotropin-releasing hormone (TRH) mRNA levels. There was no significant effect of thyroid hormone (T3) and MMI on TRH mRNA levels in the male tilapia.

(photoperiod), metabolism, and stress, that have direct or indirect impact on the kisspeptin system (48, 49), which may alter the release of GnRH.

We previously found the significant effect of thyroid hormone on *gnrh1* mRNA levels in sexually mature but not in immature tilapia (22). This result suggests that in sexually mature fish, GnRH1 neurons may acquire sensitivity to thyroid hormone due to the presence of TR, which might be absent in sexually immature fish. Similarly in male monkeys, hypothyroidism fails to prevent the arrest of GnRH pulse generator activity during the infant-juvenile transition (43). Therefore, the action of thyroid hormone on kisspeptin-GnRH neurons could be regulated in a reproductive stage-dependent manner.

EFFECT OF THYROID HORMONE ON GnRH NEURONS: DIRECT AND INDIRECT PATHWAYS

The role of thyroid hormone in reproductive functions is important during developmental as well as in the adult stages. In rats, irregular estrous cycle, failure of LH surge, impairment in male sexual behavior, and reduction of GnRH biosynthesis has been shown when hypothyroidism was induced during their adult stage (50–52). A recent study in the rat has shown the presence of type II deiodinase in GnRH neuronal axons in the median eminence as well as in GT1-7 cells (53), indicating the possible synthesis of thyroid hormone within GnRH neurons and possible direct action of thyroid hormone on GnRH neurons. In the present study, we found the expression of TR mRNA types in GnRH1 neurons, which also has been reported in the sheep and hamsters (21). The promoter region of rat GnRH gene contains motifs resembling ER/TR response elements (54). Furthermore, the rat GnRH promoter contains a retinoic acid response element (54), which can interact with TRs alone or with TR/retinoic acid receptor heterodimers (21). Therefore, thyroid hormone can directly act on GnRH1 neurons to regulate the synthesis of GnRH peptides.

In the reproductive axis, pulse, and surge pattern of GnRH secretion are critical. Recent studies in mammals have suggested that kisspeptin neurons in the arcuate nucleus (Arc) are responsible for the pulsatile release of GnRH (55). In ewes, thyroid hormones are required for steroid-independent seasonal LH pulse frequency (24), in which LH pulse frequency and amplitude alters in the absence of estradiol (56). This could be mediated through TR localized in the Arc (57) that contains kisspeptin

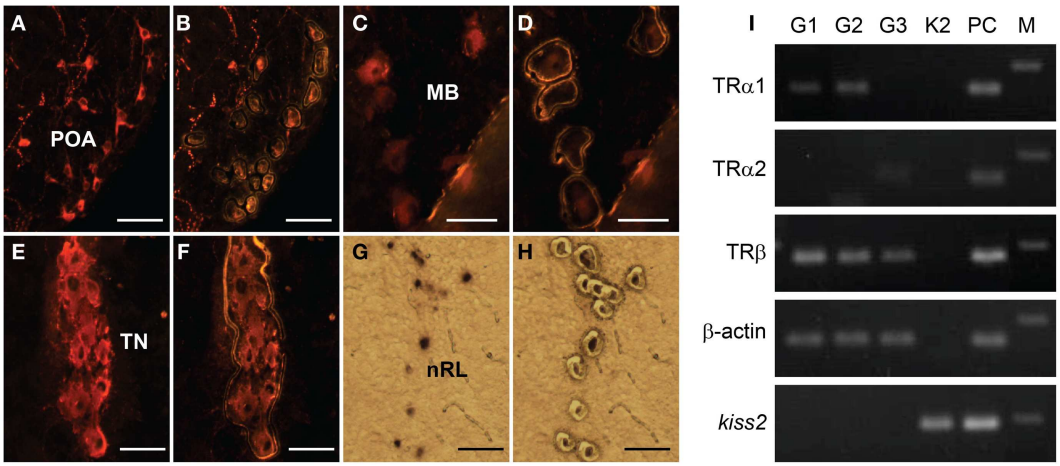

FIGURE 7 | Expression of thyroid hormone receptor (TR) types mRNA in laser-captured GnRH1, GnRH2, GnRH3, and *kiss2* neurons. **(A–H)** Photomicrographs of GnRH1 **(A,B)**, GnRH2 **(C,D)**, GnRH3-immunoreactive **(E,F),** and DIG-labeled *kiss2* **(G,H)** neurons before **(A,C,E,G)**, and after **(B,D,F,H)** laser-capture microdissection. Scale bars, 50 μm. POA, preoptic area; MB, midbrain; TN, terminal nerve; nRL, the nucleus of lateral recesses. **(I)** RT-PCR of the *tra1*, *tra2*, *trb*, β-actin, and *kiss2* genes in the laser-captured immune-fluorescently labeled three GnRH types neurons (G1, G2 and G3) and DIG-labeled *kiss2* neurons (K2). PC, Whole-brain cDNA as a positive control; M, 100-bp DNA ladder.

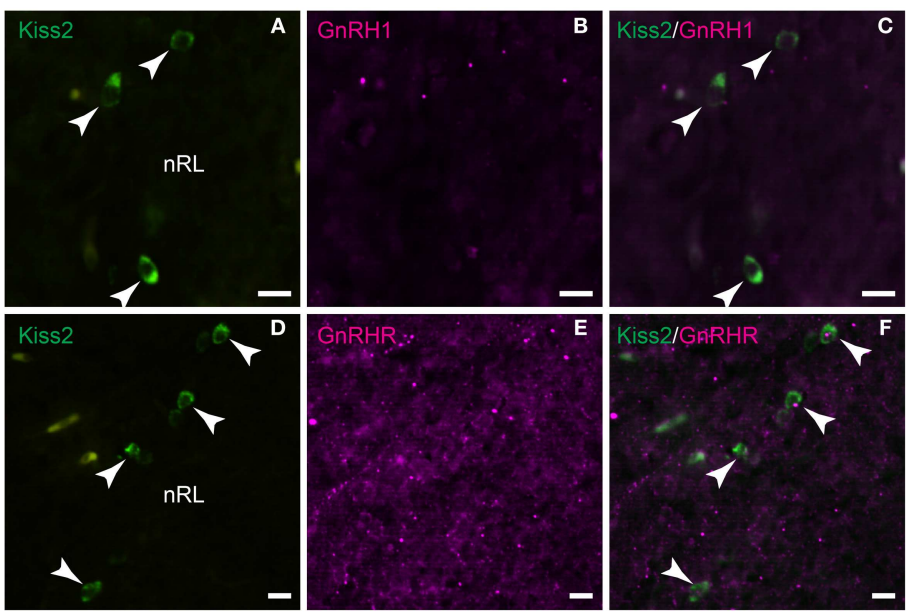

FIGURE 8 | Double-label immunofluorescence photomicrographs of tilapia *kiss2* and GnRH1 or GnRHR in the brain of male tilapia. **(A–C)** There was no GnRH1 (GAP1)-immunoreactive fibers (magenta) nearby *kiss2* mRNA containing cells (green, arrow heads) in the nucleus of lateral recesses (nRL). **(D–F)** There was no co-expression of GnRHR immunoreactivity (magenta) in *kiss2* mRNA containing cells (green, arrow heads). Scale bars, 10 μm.

neurons. Although kisspeptin has been considered a major regulator of GnRH neurons, a morphological study in the rhesus monkey has shown occasional contacts between GnRH axons and kisspeptin neurons in the Arc, indicating the possibility that GnRH could exert control over kisspeptin neuronal activity (58). However, in this study, we failed to observe any GnRH1 fibers or GnRHR in Kiss2 neurons. Therefore, it is possible that the thyroid hormone indirectly regulates Kiss2 neurons via unidentified neuronal population expressing conventional TR.

In the present study, we failed to observe the expression of TR mRNA types in Kiss2 population, which could be due to low expression levels of TR genes in *kiss2* neurons. The absence of TR does not necessarily indicate the possibility of an indirect action of thyroid hormone. Several studies have suggested the presence

of a non-classical thyroid hormone signaling pathway, which is non-genomic and does not require thyroid hormone interaction with the TR (59). In addition, *kiss2* gene could also be influenced by estrogen feedback via thyroid hormone action on the hypothalamic-pituitary-gonadal axis (60). Our recent report in goldfish showed presence of ERs in *kiss1* and *kiss2* neurons as well as activation of *kiss1* and *kiss2* gene promoters by estrogen (61). Currently we have no direct evidence of steroid sensitivity of *kiss2* gene in the tilapia, but our promoter analysis showed the presence of two possible ER response elements in the upstream of tilapia *kiss2* gene. Therefore, tilapia *kiss2* gene could also be influenced by estrogen in the male tilapia.

It is well known that TRH and thyroid-stimulating hormone (TSH) genes are regulated by thyroid hormone in mammals via negative feedback mechanism (62). However, in the few fish species studied, both T_4 and T_3 have a negative feedback effect on TSH secretion by the pituitary (63). Furthermore, it is still unknown whether T_4 or T_3 influences hypothalamic release of TRH in teleosts (60). In the present study, we failed to see any change in TRH mRNA expression by thyroid hormone manipulation. Similar observation has been reported in Senegalese sole that hormonal treatments using thiourea and T_4 showed no regulation at transcriptional levels of TRH by thyroid hormones (64) and they suggested that TRH could not participate in the hypothalamic-pituitary-thyroid axis in teleosts. This is further supported by other studies that failed to demonstrate an induction of TSH or T_4 release after TRH treatments in fish (65, 66). Therefore, in the tilapia, TRH could be insensitive to thyroid hormone levels. In addition, in mammals, not all TRH expressing neurons are T_3 responsive (67). Therefore, it is also possible that current treatment protocol (dose and duration) in this study was not sufficient enough to alter TRH mRNA levels. We noted large variation in *gnrh3* mRNA expression in the controls in the two experiments. Such variations in *gnrh3* mRNA levels have previously been reported in teleosts (68, 69), which could be due to different social and reproductive states of fish (70–72).

In summary, we cloned *kiss2* gene in the Nile tilapia. The *kiss2* mRNA was expressed in the central and peripheral tissues. DIG-*in situ* hybridization showed *kiss2* mRNA containing cells in the nRL. Thyroid hormone (T_3) treatment significantly increased *kiss2* and *gnrh1* mRNA levels, while those genes were suppressed under hypothyroid condition with MMI treatment. Presence of TR mRNA types in GnRH1 neurons and their absence in Kiss2 neurons suggest that GnRH1 may be directly regulated through thyroid hormone, while the regulation of Kiss2 by T_3 is more likely to be indirect.

AUTHORS CONTRIBUTION

Satoshi Ogawa and Ishwar S. Parhar designed the study; Satoshi Ogawa performed *in silico* gene sequence analysis, hormone treatments, data analysis, and wrote the manuscript; Kai We Ng performed cloning and real-time PCR; Xiaoyu Xue, Shuisheng Li, Berta Levavi-Sivan, Haoran Lin, Xiaochun Liu performed cloning, sequence analysis, RT-PCR; Priveena Nair Ramadasan performed *in situ* hybridization; Mageswary Sivalingam performed double-immunofluorescence and laser capture microdissection; Ishwar S. Parhar edited the manuscript, all authors approved and commented on the manuscript.

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REFERENCES

1. Tena-Sempere M. GPR54 and kisspeptin in reproduction. *Hum Reprod Update* (2006) **12**:631–9. doi:10.1093/humupd/dml023
2. Dungan HM, Clifton DK, Steiner RA. Minireview: kisspeptin neurons as central processors in the regulation of gonadotropin-releasing hormone secretion. *Endocrinology* (2006) **147**:1154–8. doi:10.1210/en.2005-1282
3. Gottsch ML, Cunningham MJ, Smith JT, Popa SM, Acohido BV, Crowley WF, et al. A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. *Endocrinology* (2004) **145**:4073–7. doi:10.1210/en.2004-0431
4. Thompson EL, Patterson M, Murphy KG, Smith KL, Dhillo WS, Todd JF, et al. Central and peripheral administration of kisspeptin-10 stimulates the hypothalamic-pituitary-gonadal axis. *J Neuroendocrinol* (2004) **16**:850–8. doi:10.1111/j.1365-2826.2004.01240.x
5. Akazome Y, Kanda S, Okubo K, Oka Y. Functional and evolutionary insights into vertebrate kisspeptin systems from studies of fish brain. *J Fish Biol* (2010) **76**:161–82. doi:10.1111/j.1095-8649.2009.02496.x
6. Um HN, Han JM, Hwang JI, Hong SI, Vaudry H, Seong JY. Molecular coevolution of kisspeptins and their receptors from fish to mammals. *Ann N Y Acad Sci* (2010) **1200**:67–74. doi:10.1111/j.1749-6632.2010.05508.x
7. Ogawa S, Parhar IS. Anatomy of the kisspeptin systems in teleosts. *Gen Comp Endocrinol* (2012) **181**:169–74. doi:10.1016/j.ygcen.2012.08.023
8. Felip A, Zanuy S, Pineda R, Pinilla L, Carrillo M, Tena-Sempere M, et al. Evidence for two distinct KiSS genes in non-placental vertebrates that encode kisspeptins with different gonadotropin-releasing activities in fish and mammals. *Mol Cell Endocrinol* (2009) **312**:61–71. doi:10.1016/j.mce.2008.11.017
9. Kitahashi T, Ogawa S, Parhar IS. Cloning and expression of *kiss2* in the zebrafish and medaka. *Endocrinology* (2009) **150**:821–31. doi:10.1210/en.2008-0940
10. Selvaraj S, Kitano H, Fujinaga Y, Ohga H, Yoneda M, Yamaguchi A, et al. Molecular characterization, tissue distribution, and mRNA expression profiles of two Kiss genes in the adult male and female chub mackerel (*Scomber japonicus*) during different gonadal stages. *Gen Comp Endocrinol* (2010) **169**:28–38. doi:10.1016/j.ygcen.2010.07.011
11. Shahjahan M, Motohashi E, Doi H, Ando H. Elevation of Kiss2 and its receptor gene expression in the brain and pituitary of grass puffer during the spawning season. *Gen Comp Endocrinol* (2010) **169**:48–57. doi:10.1016/j.ygcen.2010.07.008
12. Parhar IS, Ogawa S, Sakuma Y. Laser-captured single digoxigenin-labeled neurons of gonadotropin-releasing hormone types reveal a novel G protein-coupled receptor (Gpr54) during maturation in cichlid fish. *Endocrinology* (2004) **145**:3613–8. doi:10.1210/en.2004-0395
13. Smith JT, Clarke IJ. Kisspeptin expression in the brain: catalyst for the initiation of puberty. *Rev Endocr Metab Disord* (2007) **8**:1–9. doi:10.1007/s11154-007-9026-4
14. Mitani Y, Kanda S, Akazome Y, Zempo B, Oka Y. Hypothalamic Kiss1 but not Kiss2 neurons are involved in estrogen feedback in medaka (*Oryzias latipes*). *Endocrinology* (2010) **151**:1751–9. doi:10.1210/en.2009-1174
15. Kanda S, Karigo T, Oka Y. Steroid sensitive kiss2 neurones in the goldfish: evolutionary insights into the duplicate kisspeptin gene-expressing neurones. *J Neuroendocrinol* (2012) **24**:897–906. doi:10.1111/j.1365-2826.2012.02296.x

16. Servili A, Le Page Y, Leprince J, Caraty A, Escobar S, Parhar IS, et al. Organization of two independent kisspeptin systems derived from evolutionary-ancient kiss genes in the brain of zebrafish. *Endocrinology* (2011) **152**:1527–40. doi:10.1210/en.2010-0948
17. Clarkson J, Han S-K, Liu X, Lee K, Herbison AE. Neurobiological mechanisms underlying kisspeptin activation of gonadotropin-releasing hormone (GnRH) neurons at puberty. *Mol Cell Endocrinol* (2010) **324**:45–50. doi:10.1016/j.mce.2010.01.026
18. Bakker J, Pierman S, González-Martínez D. Effects of aromatase mutation (ArKO) on the sexual differentiation of kisspeptin neuronal numbers and their activation by same versus opposite sex urinary pheromones. *Horm Behav* (2010) **57**:390–5. doi:10.1016/j.yhbeh.2009.11.005
19. Cooke PS, Holsberger DR, Witorsch RJ, Sylvester PW, Meredith JM, Treinen KA, et al. Thyroid hormone, glucocorticoids, and prolactin at the nexus of physiology, reproduction, and toxicology. *Toxicol Appl Pharmacol* (2004) **194**:309–35. doi:10.1016/j.taap.2003.09.016
20. Cyr DG, Eales J. Interrelationships between thyroidal and reproductive endocrine systems in fish. *Rev Fish Biol Fish* (1996) **6**:165–200. doi:10.1007/BF00182342
21. Jansen HT, Lubbers LS, Macchia E, Degroot LJ, Lehman MN. Thyroid hormone receptor (α) distribution in hamster and sheep brain: colocalization in gonadotropin-releasing hormone and other identified neurons. *Endocrinology* (1997) **138**:5039–47. doi:10.1210/en.138.11.5039
22. Parhar IS, Soga T, Sakuma Y. Thyroid hormone and estrogen regulate brain region-specific messenger ribonucleic acids encoding three gonadotropin-releasing hormone genes in sexually immature male fish, *Oreochromis niloticus*. *Endocrinology* (2000) **141**:1618–26. doi:10.1210/en.141.5.1618
23. Webster JR, Moenter SM, Barrell GK, Lehman MN, Karsch FJ. Role of the thyroid gland in seasonal reproduction. III. Thyroidectomy blocks seasonal suppression of gonadotropin-releasing hormone secretion in sheep. *Endocrinology* (1991) **129**:1635–43. doi:10.1210/endo-129-1-176
24. Anderson GM, Connors JM, Hardy SL, Valent M, Goodman RL. Thyroid hormones mediate steroid-independent seasonal changes in luteinizing hormone pulsatility in the ewe. *Biol Reprod* (2002) **66**:701–6. doi:10.1095/biolreprod66.3.701
25. Keen KL, Wegner FH, Bloom SR, Ghatei MA, Terasawa E. An Increase in kisspeptin-54 release occurs with the pubertal increase in luteinizing hormone-releasing hormone-1 release in the stalk-median eminence of female rhesus monkeys *in vivo*. *Endocrinology* (2008) **149**:4151–7. doi:10.1210/en.2008-0231
26. Mol KA, van der Geyten S, Kühn ER, Darras VM. Effects of experimental hypo- and hyperthyroidism on iodothyronine deiodinases in Nile tilapia, *Oreochromis niloticus*. *Fish Physiol Biochem* (1999) **20**:201–7. doi:10.1023/A:1007739431710
27. Sukumar P, Munro AD, Mok EY, Subburaju S, Lam TJ. Hypothalamic regulation of the pituitary-thyroid axis in the tilapia *Oreochromis mossambicus*. *Gen Comp Endocrinol* (1997) **106**:73–84. doi:10.1006/gcen.1996.6852
28. Campinho MA, Morgado I, Pinto PIS, Silva N, Power DM. The goitrogenic efficiency of thioamides in a marine teleost, sea bream (*Sparus auratus*). *Gen Comp Endocrinol* (2012) **179**:369–75. doi:10.1016/j.ygcen.2012.09.022
29. Soga T, Ogawa S, Millar RP, Sakuma Y, Parhar IS. Localization of the three GnRH types and GnRH receptors in the brain of a cichlid fish: insights into their neuroendocrine and neuromodulator functions. *J Comp Neurol* (2005) **487**:28–41. doi:10.1002/cne.20519
30. van Aerle R, Kille P, Lange A, Tyler CR. Evidence for the existence of a functional Kiss1/Kiss1 receptor pathway in fish. *Peptides* (2008) **29**:57–64. doi:10.1016/j.peptides.2007.10.018
31. Li S, Zhang Y, Liu Y, Huang X, Huang W, Lu D, et al. Structural and functional multiplicity of the kisspeptin/GPR54 system in goldfish (Carassius auratus). *J Endocrinol* (2009) **201**:407–18. doi:10.1677/JOE-09-0016
32. Demski LS, Evan AP, Saland LC. The structure of the inferior lobe of the teleost hypothalamus. *J Comp Neurol* (1975) **161**:483–97. doi:10.1002/cne.901610402
33. Orsini MJ, Klein MA, Beavers MP, Connolly PJ, Middleton SA, Mayo KH. Metastin (KiSS-1) mimetics identified from peptide structure-activity relationship-derived pharmacophores and directed small molecule database screening. *J Med Chem* (2007) **50**:462–71. doi:10.1021/jm0609824
34. Kanda S, Akazome Y, Matsunaga T, Yamamoto N, Yamada S, Tsukamura H, et al. Identification of KiSS-1 product kisspeptin and steroid-sensitive sexually dimorphic kisspeptin neurons in medaka (*Oryzias latipes*). *Endocrinology* (2008) **149**:2467–76. doi:10.1210/en.2007-1503
35. Shimizu Y, Tomikawa J, Hirano K, Nanikawa Y, Akazome Y, Kanda S, et al. Central distribution of kiss2 neurons and peri-pubertal changes in their expression in the brain of male and female red seabream *Pagrus major*. *Gen Comp Endocrinol* (2012) **175**:432–42. doi:10.1016/j.ygcen.2011.11.038
36. Escobar S, Felip A, Gueguen M-M, Zanuy S, Carrillo M, Kah O, et al. Expression of kisspeptins in the brain and pituitary of the European sea bass (*Dicentrarchus labrax*). *J Comp Neurol* (2013) **521**:933–48. doi:10.1002/cne.23211
37. Lehman MN, Merkley CM, Coolen LM, Goodman RL. Anatomy of the kisspeptin neural network in mammals. *Brain Res* (2010) **1364**:90–102. doi:10.1016/j.brainres.2010.09.020
38. Denis-Donini S, Branduardi P, Campiglio S, Carnevali MDC. Localization of calcitonin gene-related peptide mRNA in developing olfactory axons. *Cell Tissue Res* (1998) **294**:81–91. doi:10.1007/s004410051158
39. Demski LS. Feeding and aggressive behavior evoked by hypothalamic stimulation in a cichlid fish. *Comp Biochem Physiol A Comp Physiol* (1973) **44**:685–92. doi:10.1016/0300-9629(73)90134-5
40. Mechaly AS, Vinas J, Piferrer F. Gene structure analysis of kisspeptin-2 (Kiss2) in the Senegalese sole (*Solea senegalensis*): characterization of two splice variants of Kiss2, and novel evidence for metabolic regulation of kisspeptin signaling in non-mammalian species. *Mol Cell Endocrinol* (2011) **339**:14–24. doi:10.1016/j.mce.2011.03.004
41. Fernandez-Fernandez R, Martini AC, Navarro VM, Castellano JM, Dieguez C, Aguilar E, et al. Novel signals for the integration of energy balance and reproduction. *Mol Cell Endocrinol* (2006) **254–255**:127–32. doi:10.1016/j.mce.2006.04.026
42. Gaytán F, Gaytán M, Castellano JM, Romero M, Roa J, Aparicio B, et al. KiSS-1 in the mammalian ovary: distribution of kisspeptin in human and marmoset and alterations in KiSS-1 mRNA levels in a rat model of ovulatory dysfunction. *Am J Physiol Endocrinol Metab* (2009) **296**:E520–31. doi:10.1152/ajpendo.90895.2008
43. Mann DR, Plant TM. The role and potential sites of action of thyroid hormone in timing the onset of puberty in male primates. *Brain Res* (2010) **1364**:175–85. doi:10.1016/j.brainres.2010.09.082
44. Yamamura T, Hirunagi K, Ebihara S, Yoshimura T. Seasonal morphological changes in the neuro-glial interaction between gonadotropin-releasing hormone nerve terminals and glial endfeet in Japanese quail. *Endocrinology* (2004) **145**:4264–7. doi:10.1210/en.2004-0366
45. Mann DR, Bhat GK, Stah CD, Pohl CR, Plant TM. Induction of a hypothyroid state during juvenile development delays pubertal reactivation of the neuroendocrine system governing luteinising hormone secretion in the male rhesus monkey (*Macaca mulatta*). *J Neuroendocrinol* (2006) **18**:662–71. doi:10.1111/j.1365-2826.2006.01460.x
46. Reddy P, Brown C, Leatherland J, Lam T. Role of thyroid hormones in tilapia larvae (*Oreochromis mossambicus*): II. Changes in the hormones and 5'-monodeiodinase activity during development. *Fish Physiol Biochem* (1992) **9**:487–96. doi:10.1007/BF02274228
47. Parhar IS. GnRH in tilapia: three genes, three origins and their roles. In: Parhar IS, Sakuma Y, editors. *GnRH Neurons: Gene to Behavior*. Tokyo: Brain Shuppan (1997). p. 99–122.
48. Castellano JM, Bentsen AH, Mikkelsen JD, Tena-Sempere M. Kisspeptins: bridging energy homeostasis and reproduction. *Brain Res* (2010) **1364**:129–38. doi:10.1016/j.brainres.2010.08.057
49. Williams WP, Jarjisian SG, Mikkelsen JD, Kriegsfeld LJ. Circadian control of kisspeptin and a gated GnRH response mediate the preovulatory luteinizing hormone surge. *Endocrinology* (2011) **152**:595–606. doi:10.1210/en.2010-0943
50. Mattheij JAM, Swarts JJM, Lokerse P, van Kampen JT, van der Heide D. Effect of hypothyroidism on the pituitary-gonadal axis in the adult female rat. *J Endocrinol* (1995) **146**:87–94. doi:10.1677/joe.0.1460087
51. Jiang J-Y, Umezu M, Sato E. Characteristics of infertility and the improvement of fertility by thyroxine treatment in adult male hypothyroid rdw rats. *Biol Reprod* (2000) **63**:1637–41. doi:10.1095/biolreprod63.6.1637
52. Toni R, Della Casa C, Castorina S, Cocchi D, Celotti F. Effects of hypothyroidism and endocrine disruptor-dependent non-thyroidal illness syndrome on

the GnRH-gonadotroph axis of the adult male rat. *J Endocrinol Invest* (2005) **28**(Suppl 11 Proceedings):20–7.
53. Kalló I, Mohácsik P, Vida B, Zeöld A, Bardóczi Z, Zavacki AM, et al. A novel pathway regulates thyroid hormone availability in rat and human hypothalamic neurosecretory neurons. *PLoS One* (2012) **7**:e37860. doi:10.1371/journal.pone.0037860
54. Kepa JK, Wang C, Neeley CI, Raynolds MV, Gordon DF, Wood WM, et al. Structure of the rat gonadotropin releasing hormone (rGnRH) gene promoter and functional analysis in hypothalamic cells. *Nucleic Acids Res* (1992) **20**:1393–9. doi:10.1093/nar/20.6.1393
55. Navarro VM, Gottsch ML, Chavkin C, Okamura H, Clifton DK, Steiner RA. Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. *J Neurosci* (2009) **29**:11859–66. doi:10.1523/JNEUROSCI.1569-09.2009
56. Robinson JE, Radford HM, Karsch FJ. Seasonal changes in pulsatile luteinizing hormone (LH) secretion in the ewe: relationship of frequency of LH pulses to day length and response to estradiol negative feedback. *Biol Reprod* (1985) **33**:324–34. doi:10.1095/biolreprod33.2.324
57. Alkemade A, Vuijst CL, Unmehopa UA, Bakker O, Vennström B, Wiersinga WM, et al. Thyroid hormone receptor expression in the human hypothalamus and anterior pituitary. *J Clin Endocrinol Metab* (2005) **90**:904–12. doi:10.1210/jc.2004-0474
58. Ramaswamy S, Guerriero KA, Gibbs RB, Plant TM. Structural interactions between kisspeptin and GnRH neurons in the mediobasal hypothalamus of the male rhesus monkey (*Macaca mulatta*) as revealed by double immunofluorescence and confocal microscopy. *Endocrinology* (2008) **149**:4387–95. doi:10.1210/en.2008-0438
59. Cordeiro A, Souza LL, Einicker-Lamas M, Pazos-Moura CC. Non-classic thyroid hormone signalling involved in hepatic lipid metabolism. *J Endocrinol* (2013) **216**(3):R47–57. doi:10.1530/JOE-12-0542
60. Blanton ML, Specker JL. The hypothalamic-pituitary-thyroid (HPT) axis in fish and its role in fish development and reproduction. *Crit Rev Toxicol* (2007) **37**:97–115. doi:10.1080/10408440601123529
61. Wang Q, Sham KWY, Ogawa S, Li S, Parhar IS, Cheng CHK, et al. Regulation of the two kiss promoters in goldfish (*Carassius auratus*) by estrogen via different ERα pathways. *Mol Cell Endocrinol* (2013) **375**:130–9. doi:10.1016/j.mce.2013.04.023
62. Hollenberg AN, Monden T, Flynn TR, Boers ME, Cohen O, Wondisford FE. The human thyrotropin-releasing hormone gene is regulated by thyroid hormone through two distinct classes of negative thyroid hormone response elements. *Mol Endocrinol* (1995) **9**:540–50. doi:10.1210/me.9.5.540
63. Yoshiura Y, Sohn Y, Munakata A, Kobayashi M, Aida K. Molecular cloning of the cDNA encoding the β subunit of thyrotropin and regulation of its gene expression by thyroid hormones in the goldfish, *Carassius auratus*. *Fish Physiol Biochem* (1999) **21**:201–10. doi:10.1023/A:1007884527397
64. Iziga R, Ponce M, Infante C, Rebordinos L, Cañavate JP, Manchado M. Molecular characterization and gene expression of thyrotropin-releasing hormone in Senegalese sole (*Solea senegalensis*). *Comp Biochem Physiol B Biochem Mol Biol* (2010) **157**:167–74. doi:10.1016/j.cbpb.2010.05.013
65. Melamed P, Eliahu N, Levavi-Sivan B, Ofir M, Farchi-Pisanty O, Rentier-Delrue F, et al. Hypothalamic and thyroidal regulation of growth hormone in tilapia. *Gen Comp Endocrinol* (1995) **97**:13–30. doi:10.1006/gcen.1995.1002
66. Larsen DA, Swanson P, Dickey JT, Rivier J, Dickhoff WW. In vitro thyrotropin-releasing activity of corticotropin-releasing hormone-family peptides in coho salmon, *Oncorhynchus kisutch*. *Gen Comp Endocrinol* (1998) **109**:276–85. doi:10.1006/gcen.1997.7031
67. Sugrue ML, Vella KR, Morales C, Lopez ME, Hollenberg AN. The thyrotropin-releasing hormone gene is regulated by thyroid hormone at the level of transcription in vivo. *Endocrinology* (2010) **151**:793–801. doi:10.1210/en.2009-0976
68. Parhar IS, Ogawa S, Hamada T, Sakuma Y. Single-cell real-time quantitative polymerase chain reaction of immunofluorescently identified neurons of gonadotropin-releasing hormone subtypes in cichlid fish. *Endocrinology* (2003) **144**:3297–300. doi:10.1210/en.2003-0386
69. Levy G, Gothilf Y, Degani G. Brain gonadotropin releasing hormone3 expression variation during oogenesis and sexual behavior and its effect on pituitary hormonal expression in the blue Gourami. *Comp Biochem Physiol A Mol Integr Physiol* (2009) **154**:241–8. doi:10.1016/j.cbpa.2009.06.010
70. Oka Y. Gonadotropin-releasing hormone (GnRH) cells of the terminal nerve as a model neuromodulator system. *Neurosci Lett* (1992) **142**:119–22. doi:10.1016/0304-3940(92)90353-9
71. Yamamoto N, Oka Y, Kawashima S. Lesions of gonadotropin-releasing hormone-immunoreactive terminal nerve cells: effects on the reproductive behavior of male Dwarf gouramis. *Neuroendocrinology* (1997) **65**:403–12. doi:10.1159/000127203
72. Ogawa S, Soga T, Sakuma Y, Parhar IS. Modulation of GnRH subtypes by social stress and aggressive behavior. *Fish Physiol Biochem* (2003) **28**:49–50. doi:10.1023/B:FISH.0000030474.32151.12

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