Thyroid hormone is classically known to play a crucial role in neurodevelopment. The potent effects that thyroid hormone exerts on the adult mammalian brain have been uncovered relatively recently, including an important role in the modulation of progenitor development in adult neurogenic niches. This chapter extensively reviews the current understanding of the influence of thyroid hormone on distinct stages of adult progenitor development in the subgranular zone (SGZ) of the hippocampus and...
subventricular zone (SVZ) that lines the lateral ventricles. We discuss the role of specific thyroid hormone receptor isoforms, in particular TRα1, which modulates cell cycle exit in neural stem cells, progenitor survival, and cell fate choice, with both a discrete and overlapping nature of regulation noted in SGZ and SVZ progenitors. The balance between liganded and unliganded TRα1 can evoke differing consequences for adult progenitor development, and the relevance of this to conditions such as adult-onset hypothyroidism, wherein unliganded thyroid hormone receptors (TRs) dominate, is also a focus of discussion. Although a detailed molecular understanding of the specific thyroid hormone target genes that contribute to the neurogenic actions of thyroid hormone is currently lacking, we highlight the current state of knowledge and discuss avenues for future investigation. The goal of this chapter is to provide a comprehensive and detailed analysis of the effects of thyroid hormone on adult neurogenesis, to discuss putative molecular mechanisms that mediate these effects, and the behavioral, functional, and clinical implications of the neurogenic actions of thyroid hormone.

Thyroid hormone plays a seminal role in shaping the development of the mammalian nervous system (Bernal, 2007; Preau, Fini, Morvan-Dubois, & Demeneix, 2015; Rovet, 2014). While the role of thyroid hormone has been best appreciated and studied during neurodevelopment, a growing body of knowledge also highlights its continued role in the regulation of plasticity within the adult mammalian brain (Bauer, Goetz, Glenn, & Whybrow, 2008; Calza, Aloe, & Giardino, 1997; Koromilas et al., 2010; Sarkar, 2002). The critical influence of thyroid hormone during neurodevelopment was the primary focus of several studies due to the clinical observations that highlighted the severe neurological consequences of perturbations of euthyroid status during gestational and early postnatal development (de Escobar, Obregon, & del Rey, 2007; Dugbartey, 1998; Williams, 2008; Zoeller & Crofton, 2005). Relatively few studies focussed on a continued role for thyroid hormone in the regulation of adult brain structure, plasticity, and function, only further contributing to the common idea that the effects of thyroid hormone on the mammalian brain are largely restricted to “critical periods” of neurodevelopment (Axelstad et al., 2008; Bernal, 2002; Gilbert & Sui, 2006; Mastorakos, Karoutou, Mizamtsidi, & Creatas, 2007; Zoeller & Rovet, 2004). This perception has now been revised, due to several studies that highlight the important role of thyroid hormone within the adult brain (Henley & Koehnle, 1997; Koromilas et al., 2010; Schroeder & Privalsky, 2014), in particular, a key influence of thyroid hormone in the regulation of progenitor development within
the neurogenic niches of the adult brain (Kapoor, Fanibunda, Desouza, Guha, & Vaidya, 2015; Remaud, Gothie, Morvan-Dubois, & Demeneix, 2014). The focus of this chapter is to review in detail the literature focussed on the influence of thyroid hormone on adult mammalian neurogenesis, and to discuss the functional implications of the neurogenic effects of thyroid hormone.

1. THYROID HORMONE AND THE ADULT BRAIN

The synthesis of thyroid hormone is under the control of the hypothalamic–pituitary–thyroid axis. The hypothalamus secretes thyrotropin-releasing hormone (TRH), which in turn stimulates the release of thyroid-stimulating hormone (TSH) from the pituitary. TSH results in the synthesis and secretion of thyroid hormone from the follicular cells of the thyroid gland (Chiamolera & Wondisford, 2009; Costa-e-Sousa & Hollenberg, 2012; Joseph-Bravo, Jaimes-Hoy, Uribe, & Charli, 2015). The secreted thyroid hormone is largely in the thyroxine (3,3',5,5'-tetraiodothyronine, T4) precursor form and in smaller quantities in its active form (3,3',5-triiodothyronine, T3) and is transported in the plasma bound to passive carriers, including albumin, thyroxine-binding globulin, and transthyretin (TTR). At the blood–brain barrier, the active entry of thyroid hormone into the brain is facilitated via TTR in the choroid plexus and the organic anion transporter proteins (Bernal, Guadano-Ferraz, & Morte, 2015; Jansen, Friesema, Milici, & Visser, 2005; Richardson, Wijayagumaratne, D'Souza, Darras, & Van Herck, 2015; Wirth, Schweizer, & Kohrle, 2014). On entry into the brain, the local availability of thyroid hormone is further modulated by the activity of three types of iodothyronine deiodinases—I, II, and III (D1, D2, and D3). In particular, coordination of the relative expression and activity of D2, which converts the prohormone T4 to active T3, and D3, which is responsible for inactivation of T3 and T4, can dynamically modulate the levels of thyroid hormone available within local brain regions (Bianco, 2011; Bianco & Kim, 2006).

Thyroid hormone mediates its actions through thyroid hormone receptors (TRs), which act as transcription factors to regulate gene expression of target genes (Cheng, Leonard, & Davis, 2010; Harvey & Williams, 2002). Alternative splicing of two TR genes—alpha and beta—results in multiple different TR isoforms that exhibit considerable similarity in their DNA-binding domains, but differ in their transactivation and ligand-
binding domains. The TRα1, TRα2, TRβ1, and TRβ2 isoforms are expressed in the mammalian brain (Bradley, Towle, & Young, 1992; Forrest & Vennstrom, 2000; Mellstrom, Naranjo, Santos, Gonzalez, & Bernal, 1991), with TRα1 accounting for 70% of the total TR expression (Schwartz, Strait, Ling, & Oppenheimer, 1992; Wallis et al., 2010). The TRα2 isoform due to an absence of thyroid hormone-binding ability is suggested to exert a dominant-negative role via thyroid hormone response elements (TREs) within target genes (Guissouma et al., 2014; Koenig et al., 1989). Of the TRβ isoforms, TRβ1 is widely expressed across the brain (Forrest & Vennstrom, 2000; Murata, 1998; Zhang & Lazar, 2000), while TRβ2 expression is limited to the hypothalamus and pituitary (Abel, Ahima, Boers, Elmquist, & Wondisford, 2001; Cook, Kakucska, Lechan, & Koenig, 1992; Lechan, Qi, Jackson, & Mahdavi, 1994), with expression levels increasing across postnatal development. The expression of thyroid hormone target genes can be regulated by both ligand-bound TRs as well as unliganded aporeceptors, often in a very distinct fashion (Bernal & Morte, 2013; Chassande, 2003; Zhang & Lazar, 2000). In general, the presence of thyroid hormone results in the recruitment of coactivators that mediate the acetylation of histones, and subsequent binding of RNA Polymerase II (Bernal et al., 2015; Cheng et al., 2010; Harvey & Williams, 2002; Liu & Brent, 2010). In contrast, the absence of thyroid hormone resulting in a TR aporeceptor form often causes the repression of thyroid hormone target genes due to the recruitment of corepressors that contribute to histone deacetylation (Astashova & Hollenberg, 2013; Flamant, Gauthier, & Samarut, 2007; Hu & Lazar, 2000; Jepsen & Rosenfeld, 2002; Privalsky, 2004; Shi, 2009). In addition, to the genomic effects of thyroid hormone mediated via the TRs, thyroid hormone can also exert nongenomic actions mediated by membrane-bound receptors or through modulation of signaling pathways (Cao, Kambe, Moeller, Refetoff, & Seo, 2005; Davis, Leonard, & Davis, 2008; Davis et al., 2010; Furuya, Lu, Guigon, & Cheng, 2009; Lanni, Moreno, & Goglia, 2016; Silva, Giannocco, Santos, & Nunes, 2006). The effects of thyroid hormone on the adult mammalian brain are subject to regulation at diverse levels. These span from changes in local availability of thyroid hormone through the regulation of transport and the enzymatic activity of different deiodinases, the differential expression of TR isoforms, the interaction of TRs with coactivator or corepressor complexes and chromatin remodeling machinery, the balance of ligand-dependent and independent changes in gene expression, as well as the nongenomic actions of thyroid hormone.
2. MAKING NEW NEURONS IN THE ADULT BRAIN: A ROLE FOR THYROID HORMONE

The two predominant sites within the adult mammalian brain which house the progenitors that give rise to new neurons include the subgranular zone (SGZ) in the dentate gyrus (DG) subfield of the hippocampus, and the subventricular zone (SVZ) that lines the lateral ventricles (Fig. 1).

Fig. 1 Adult neurogenesis within the major neurogenic niches of the mammalian brain. (A) Shown is a schematic depicting a sagittal section through an adult rodent brain highlighting the major neurogenic niches of (B) the subventricular zone (SVZ) lining the lateral ventricles (LV) from which progenitors traverse along the rostral migratory stream (RMS) to the olfactory bulb (OB) and (C) the subgranular zone (SGZ) in the dentate gyrus (DG) subfield of the hippocampus (Hpc). (B) The illustration highlights the distinct stages of progression for SVZ progenitors and the markers that characterize specific stages of SVZ progenitor development, starting with the Type B quiescent radial glia-like stem cell, the Type C transit amplifying progenitors progressing to the Type A migratory neuroblasts that proceed along the RMS to the OB. (C) The schematic highlights the stages of SGZ progenitor and markers associated with specific stages in the hippocampal neurogenic niche. Shown are the Type 1 quiescent neural progenitors which self-renew and also generate Type 2a amplifying neural progenitors which give rise to Type 2b cells that further mature through the Type 3 neuroblast stage to then become immature and finally mature neurons within the granule cell layer (GCL) of the DG hippocampal subfield. Shown is the expression of the stage-specific markers associated with progenitor development in both the SVZ and SGZ.
These progenitors retain the capacity to divide and give rise to daughter cells that undergo structural and functional maturation, eventually integrating into mature neuronal networks (Sailor, Schinder, & Lledo, 2016). Progenitor development and maturation are subject to regulation by diverse intrinsic and extrinsic factors at all the stages that the progenitor traverses en route to forming a mature neuron. Among the extrinsic factors, adult neurogenesis is highly sensitive to hormones, neurotransmitters, and growth factors present in the neurogenic niche (Mahmoud, Wainwright, & Galea, 2016; Perez-Domper, Gradari, & Trejo, 2013; Vaidya, Vadodaria, & Jha, 2007). Thyroid hormone has been shown to exert a strong influence on the development of progenitors both within the SGZ as well as the SVZ (Kapoor et al., 2015; Remaud et al., 2014). These studies have examined direct effects of thyroid hormone on neuronal progenitors in vitro (Desouza et al., 2005; Kapoor, Desouza, Nanavaty, Kernie, & Vaidya, 2012), as well as the consequences of perturbing the levels of circulating thyroid hormone in vivo using pharmacological and surgical approaches (Ambrogini et al., 2005; Desouza et al., 2005; Lemkine et al., 2005; Montero-Pedrazuela et al., 2006). More recently, studies have also capitalized on the use of TR isoform–specific mutant mouse lines to examine effects on adult neurogenesis (Kapoor, Ghosh, Nordstrom, Vennstrom, & Vaidya, 2011; Kapoor et al., 2010; Lemkine et al., 2005; Lopez-Juarez et al., 2012). We have a much better understanding of the progenitor stage-specific effects of thyroid hormone, although the molecular mechanisms that underlie these neurogenic effects and the target genes, both at the level of the progenitors and through influences within the neurogenic niche, remain poorly elucidated. In addition, the physiological and behavioral consequences of the neurogenic effects of thyroid hormone remain to be determined. We will describe at length the effects of thyroid hormone on both hippocampal and SVZ progenitors, highlighting the insights currently available based on the existent scientific literature and also point out lacunae in our current understanding.

3. THYROID HORMONE AND ADULT HIPPOCAMPAL NEUROGENESIS

3.1 From Hippocampal Progenitors to Mature Neurons

The entire process of generation of mature granule cell neurons within the hippocampal DG subfield from progenitors that lie within the SGZ and their functional integration into the existing neurocircuitry takes around
4–6 weeks (Goncalves, Schafer, & Gage, 2016; Ming & Song, 2011). Around 9000 progenitors have been estimated to be generated everyday within the rodent SGZ; however, only around half of these actually go on to survive and contribute to new neuron addition to the granule cell layer (GCL). The surviving progenitors are largely committed to take on the identity of excitatory granule cell neurons, with a minor fraction observed to acquire a glial fate. The milestones that a hippocampal progenitor progresses through on its journey from a neural stem cell to a mature granule cell neuron are characterized by the presence of specific markers (Fig. 1) (Kempermann, Jessberger, Steiner, & Kronenberg, 2004; Kempermann, Song, & Gage, 2015; Kuhn, Eisch, Spalding, & Peterson, 2016). The stem cell/quiescent neural progenitor (QNP) or Type 1 cell is a radial glial-like cell that expresses the intermediate filament protein nestin, the glial fibrillary acidic protein (GFAP), and the transcription factor Sox2. QNPs exhibit distinct characteristics, including the fact that they undergo asymmetric, slow division replenishing their own pool and simultaneously generating daughter cells characterized by the ability to rapidly amplify their numbers. These highly mitotic, transit amplifying neural progenitors (ANPs) also called Type 2a cells continue to express nestin, but lose GFAP and Sox2 expression. The ANPs divide rapidly to generate Type 2b neuroblasts with limited proliferative capacity, which retain nestin immunopositivity. These Type 2b hippocampal progenitors acquire expression of the microtubule-associated protein doublecortin (DCX), as well as the basic helix–loop–helix transcription factor NeuroD, that contributes to neuronal cell fate determination. Type 2b cells then go on to migrate into the GCL and undergo maturation to form DCX-positive, nestin-negative postmitotic Type 3 cells that also express polysialylated neural cell adhesion molecule (PSA-NCAM) and markers such as Stathmin and TUC-4 (Fig. 1) (Kempermann et al., 2004; Kuhn et al., 2016; Ming & Song, 2011; Zhao, Deng, & Gage, 2008). As these immature neurons undergo maturation, they extend their dendritic arbors, receive synaptic inputs, send out axonal projections to the CA3 hippocampal subfield, and undergo a switch from the transient expression of the calcium–binding protein calretinin to the expression of calbindin, which is associated with mature granule cell neurons. This entire process of maturation from a hippocampal progenitor to an integrated mature neuron takes about 4–6 weeks and is highly sensitive to perturbations of the environment both external and within the neurogenic niche (Kempermann et al., 2015; Ming & Song, 2011). During this process, immature neurons that exhibit distinct electrophysiological characteristics of high excitability and a lower
threshold for firing can disproportionately influence hippocampal network function (Song, Christian, Ming, & Song, 2012), though the total numbers of new neurons remain a relatively small fraction of all granule cell neurons. The process of adult hippocampal neurogenesis has been linked to hippocampal dependent functions such as pattern separation, spatial learning and memory, and the modulation of mood and anxiety (Besnard & Sahay, 2016; Hage & Azar, 2012; Lieberwirth, Pan, Liu, Zhang, & Wang, 2016; Miller & Hen, 2014; Sahay et al., 2011).

3.2 Thyroid Hormone Perturbations and Adult Hippocampal Neurogenesis: Insights From in vivo Studies

The influence of thyroid hormone on adult hippocampal neurogenesis was first demonstrated in rodent models (Fig. 2). Hypothyroid status was induced using goitrogen treatment or thyroidectomy, and hyperthyroidism through the administration of exogenous thyroid hormone (Ambrogini et al., 2005; Desouza et al., 2005; Montero-Pedrazuela et al., 2006). Adult-onset hypothyroidism in rats significantly decreased the postmitotic survival and neuronal differentiation of hippocampal progenitors, without affecting their proliferation (Ambrogini et al., 2005; Desouza et al., 2005). This reduction in progenitor survival was likely mediated through increased apoptotic cell death. Both the decline in progenitor survival and neuronal differentiation were normalized in hypothyroid animals by restoration of euthyroid status through thyroid hormone replacement therapy (Desouza et al., 2005). Another report in rats indicated delayed neuronal morphological maturation and the prolonged expression of the immature neuronal marker TUC-4, following goitrogen-mediated hypothyroidism (Ambrogini et al., 2005). While both these studies using goitrogens in adult rats did not alter hippocampal progenitor proliferation, findings from a study in thyroidectomized rats demonstrated a decrease in progenitor proliferation in the hippocampus (Montero-Pedrazuela et al., 2006). There was also a decrease in DCX-positive immature neurons and decreased dendritic complexity noted in this study. Interestingly, these hypothyroid effects correlated with a depressive phenotype in the forced swim test without altering cognitive performance in the novel object recognition test.

While all the studies detailed earlier were in agreement about the hypothyroidism induced decrease in hippocampal neurogenesis, the specific progenitor stages that are affected by alterations in thyroid hormone remained
unclear. Experiments by Ambrogini et al. (2005) and Desouza et al. (2005) suggested that hippocampal progenitor survival and their differentiation into neurons are affected by perturbations of thyroid hormone status. In contrast, studies by Montero-Pedrazuela et al. (2006) suggested that the proliferative stage of hippocampal progenitors is also sensitive to thyroid hormone perturbations. These discrepancies are possibly due to the differences in treatment paradigms as well as methods used to perturb thyroid hormone status. In addition, the use of bromodeoxyuridine (BrdU) to assess progenitor turnover is complicated by the fact that the BrdU administration paradigm and the time of sacrifice can result in the labeling of multiple stages of progenitor
development, making it difficult to delineate effects on cell turnover, cell cycle duration, and short-term survival (Taupin, 2007). The advent of transgenic mouse models and a better understanding of the markers that distinguish individual stages that the hippocampal progenitors traverse during their maturation process have greatly helped the study of effects on specific stages of hippocampal progenitor development. For example, the use of transgenic Nestin-green fluorescent protein (GFP) reporter mice (Yu, Dandekar, Monteggia, Parada, & Kernie, 2005) along with triple immunofluorescence labeling has made it possible to distinguish between the first three stages (Type 1, 2a, and 2b) of hippocampal progenitor development. Using these Nestin-GFP mice, studies have demonstrated that while adult-onset hypothyroidism did not affect the total proliferative pool (Type 1 and 2a) of hippocampal progenitors, there was a significant decrease in the number of Nestin-GFP and DCX double-labeled cells (Type 2b neuroblasts). In addition, the postmitotic survival of these hippocampal progenitors (Type 3, namely those that were DCX- and NeuroD-positive) was significantly decreased in adult-onset hypothyroid Nestin-GFP mice (Kapoor et al., 2012). Recent evidence also corroborates the view that it is predominantly postmitotic hippocampal progenitors that are sensitive to a decline in thyroid hormone levels, with no change in proliferation (Sanchez-Huerta, Garcia-Martinez, Vergara, Segovia, & Pacheco-Rosado, 2016) with results suggestive of an important role for thyroid hormone in maintenance and differentiation of the progenitor pool once they exit the cell cycle (Fig. 2). A recent report, using a model for moderate adult-onset thyroid hormone insufficiency, noted no effects on numbers of DCX-positive immature neurons, suggesting that the degree of thyroid hormone insufficiency may also be a critical factor in determining the extent of regulation of adult hippocampal neurogenesis (Gilbert, Goodman, Gomez, Johnstone, & Ramos, 2016).

In contrast to the clear similarities in observations from studies of the effects of adult-onset hypothyroidism in rat and mouse models, adult-onset hyperthyroidism evokes distinct effects on hippocampal neurogenesis in these rodent models. While, in rat models, increased circulating thyroid hormone levels had no effect on hippocampal progenitor proliferation, survival, or differentiation (Desouza et al., 2005), in adult-onset hyperthyroid mice a significant increase was noted in postmitotic survival and differentiation (Kapoor et al., 2012) (Fig. 2). Adult mice administered thyroid hormone exhibited significant increases in DCX- and NeuroD-positive hippocampal progenitors (Kapoor et al., 2012). Further, studies using
Nestin-GFP mice demonstrated that the maturation of hippocampal progenitors into neurons was accelerated with both a total increase noted in the number of DCX-positive (Type 3) immature neurons and a speeding up of the acquisition of markers of differentiation by hippocampal progenitors observed using a BrdU pulse-chase experiment (Kapoor et al., 2012). The differences noted in the effects of exogenous thyroid hormone treatment on adult hippocampal progenitors in the mouse vs rat brain raise certain possibilities. Cells within the hippocampal neurogenic niche or progenitor TRs in the murine brain may not be saturated under euthyroid conditions, thus retaining the possibility for additional effects (enhanced survival, accelerated maturation) upon exogenous thyroid hormone administration. In contrast, the studies thus far suggest that in case of the rat hippocampal neurogenic niche or progenitors, it is possible that TRs are already completely saturated under the baseline state of euthyroidism, leaving no scope for further effects on progenitor survival and differentiation on addition of thyroid hormone.

### 3.3 Influence of Thyroid Hormone on Hippocampal Progenitors In Vitro

While in vivo studies clearly indicate an important influence of thyroid hormone on adult hippocampal neurogenesis (Kapoor et al., 2015; Remaud et al., 2014), thus far these studies do not allow a clear distinction of whether the neurogenic effects of thyroid hormone are mediated directly on progenitors or via an influence on the neurogenic niche. In this regard, studies that have examined the influence of thyroid hormone on hippocampal progenitors in culture provide specific insights and also highlight avenues for future investigation.

Thyroid hormone treatment of dispersed hippocampal progenitor cultures indicates effects on enhanced progenitor proliferation and survival, as well as a shift toward increased glial differentiation (Desouza et al., 2005). The decline in progenitor cell death in vitro is consistent with the improved survival of DCX-positive progenitors noted upon exogenous thyroid administration in vivo. In contrast, both the enhanced proliferation and glial differentiation are only observed in vitro, suggesting that removal of hippocampal progenitors from their neurogenic niche may also alter the nature of regulation in response to thyroid hormone (Desouza et al., 2005). In an alternate in vitro model for neural stem cells, namely the hippocampal neurosphere assay, thyroid hormone treatment induced no change in the number of neurospheres, but a shift was noted from larger
to smaller neurospheres, suggesting the possibility of enhanced differentiation. Indeed, this was confirmed by the greater expression of the neuronal marker, Tuj-1, in neurospheres treated with thyroid hormone (Kapoor et al., 2012). While dispersed hippocampal progenitors and neurosphere cultures both appear to express the different TR isoforms (Desouza et al., 2005; Kapoor et al., 2012), their relative abundance is yet unknown and the presence of thyroid hormone transporters and the deiodinases has not been examined.

Although there is a certain degree of overlap between the effects of thyroid hormone observed in vitro and in vivo, in particular the enhanced progenitor survival, the range of effects in vivo suggests an important influence mediated via the neurogenic niche. This opens up an important area for investigation to delineate direct effects mediated on hippocampal progenitors and those that involve cellular components of the neurogenic niche. Within the neurogenic niche, thyroid hormone may exert an influence on neurons, astrocytes, as well as microglia, which in turn may then modulate hippocampal progenitor development and maturation. In this regard, astrocytes are of particular interest as they are known to provide important cues for progenitor development (Ashton et al., 2012; Magnusson & Frisen, 2016; Seri, Garcia-Verdugo, McEwen, & Alvarez-Buylla, 2001). While the effects of thyroid hormone on astrocytes are well studied (Dezonne, Lima, Trentin, & Gomes, 2015; Martinez & Gomes, 2002; Mohacsik, Zeold, Bianco, & Gereben, 2011; Morte & Bernal, 2014) and thyroid hormone levels are known to be locally regulated by D2 present in astrocytes (Mohacsik et al., 2011; Morte & Bernal, 2014), the contribution of astrocytes in mediating the neurogenic effects of thyroid hormone remains unclear. Thyroid hormone could exert effects on hippocampal progenitors by modulating paracrine release of growth factors such as basic fibroblast growth factor and neurotrophin-3 from astrocytes (Igelhorst, Niederkinkhaus, Karus, Lange, & Dietzel, 2015; Niederkinkhaus, Marx, Hoffmann, & Dietzel, 2009; Tseng et al., 2006). In addition, thyroid hormone also influences the regulation of diverse signaling pathways within the hippocampus. For example, thyroid hormone has been reported to regulate the expression of the developmental signaling morphogen, sonic hedgehog, and its receptors, which may in turn be relevant to the neurogenic actions of thyroid hormone (Desouza et al., 2011). Further, thyroid hormone is known to exert nongenomic effects on critical signaling pathways such as the MAP kinase cascade (Bitiktas et al., 2016), in diverse cell types including in microglia
influencing their migration and phagocytosis (Mori et al., 2015). Although
the contributions of these effects of thyroid hormone on neurons and
glia in the hippocampus in mediating the neurogenic effects of thyroid
hormone remain poorly understood, these studies motivate future research
to determine the effects of thyroid hormone that arise through direct
regulation of hippocampal progenitors and those mediated by components
of the neurogenic niche.

3.4 Contribution of TRs to Adult Hippocampal Neurogenesis

Thus far, studies have identified the postmitotic Type 2b and 3 hippocampal
progenitor stages as the critical stages of progenitor development that are
particularly responsive to thyroid hormone-mediated regulation. These pre-
dominantly postmitotic stages in the developmental progression for hippo-
campal progenitors in some sense can be viewed as a gate at which specific
signals and cues may serve to determine whether these cells survive and dif-
ferentiate, thus resulting in their integration into the hippocampal network
or end up as slated for death (Fig. 3) (Kapoor et al., 2012). In this regard,
thyroid hormone has been speculated to be such a putative cue that may
determine the cell cycle exit, eventual survival, and differentiation of adult
hippocampal progenitors. This notion is inspired by several studies with
other progenitor types, namely, oligodendrocytic progenitors, embryonic
stem cells, and myogenic progenitors wherein a role for thyroid hormone
has been identified as a key timing switch to initiate cell cycle exit and pro-
mote differentiation (Baas et al., 1997; Billon, Jolicoeur, Tokumoto,
Vennstrom, & Raff, 2002; Chen et al., 2012; Daury et al., 2001;
Fernandez, Pirondi, Manservigi, Giardino, & Calza, 2004; Pascual &
Aranda, 2013). Studies with whole-body TR isoform–specific knockout
and transgenic mice (Kapoor et al., 2012, 2011, 2010) have provided insights
into the role of thyroid hormone in hippocampal progenitor development;
at the same time the findings of these studies have also clearly highlighted the
need and importance for future experiments using conditional TR mutant
mice to gain a deeper understanding of the role of TRs in adult hippocampal
neurogenesis.

The contribution of TRα1, the dominant TR isoform within the
mammalian brain (Schwartz et al., 1992; Wallis et al., 2010), in the regula-
tion of adult hippocampal neurogenesis has been analyzed through the
use of specific mutant mouse lines namely, the TRα1−/− mice which
lack TRα1 expression, TRα2−/− mice which demonstrate a compensatory
overexpression of TRα1, the TRα1+/m heterozygous mice harboring a point mutation (TRα1R384C) in TRα1, resulting in a 10-fold lower affinity of the receptor for the ligand, and the TRα1-GFP knockin mouse line (Kapoor et al., 2010; Salto et al., 2001; Tinnikov et al., 2002; Wallis et al.,...
TRα1−/−null mice exhibited an enhanced postmitotic survival of hippocampal progenitors (Kapoor et al., 2010), a phenotype that overlaps with changes noted in adult-onset hyperthyroid mice (Kapoor et al., 2012). In contrast, TRα2−/− mice (Salto et al., 2001) that overexpress the TRα1 receptor several fold exhibited a decrease in the survival of progenitors with a decline also noted in the NeuroD-positive pool of progenitors (Kapoor et al., 2010). These observations have contributed to the hypothesis that an overexpression of TRα1 may result in a shift in balance toward an aporeceptor state, paralleling the state that arises in adult-onset hypothyroidism (Ambrogini et al., 2005; Desouza et al., 2005; Montero-Pedrazuela et al., 2006), of a shift toward unliganded TRs; and that TRα1 aporeceptor bias may then predispose postmitotic hippocampal progenitors toward cell death (Fig. 3). Further evidence in support of this hypothesis stems from studies using the dominant-negative TRα1+/m mouse line (Tinnikov et al., 2002) that harbor a mutant TRα1 (point mutation—TRα1R384C) with a 10-fold lower binding affinity for thyroid hormone, thus biasing toward a TRα1 aporeceptor form. TRα1+/m mice also exhibited a significant decline in hippocampal progenitor survival and neuronal differentiation (Kapoor et al., 2010). The decreased progenitor survival and neuronal differentiation noted in both these mutant mouse lines that cause a shift toward enhanced TRα1 aporeceptor activity, namely the TRα2−/− and TRα1+/m mice, thus recapitulating a hypothyroid-like state (Desouza et al., 2005; Kapoor et al., 2012), could be completely rescued to wild-type levels of adult neurogenesis simply through a rescue of circulating thyroid hormone levels via T3 administration (Kapoor et al., 2010). Taken together, these studies indicate that an unliganded aporeceptor TRα1 may contribute to the deficits in hippocampal neurogenesis observed following adult-onset hypothyroidism and raise the possibility that this may contribute to the cognitive and mood-related disturbances that arise due to such disruptions of euthyroid status.

While in vitro studies suggest that TRα1 is expressed by proliferating progenitors, based on qPCR and antibody staining in both dispersed hippocampal progenitor cultures and neurospheres (Desouza et al., 2005; Kapoor et al., 2012), in vivo results suggest a differential distribution of TR isoforms across progenitor development (Desouza et al., 2005). Given the paucity of high-quality antibodies that allow the ease of distinction across different TR isoforms, thus far expression studies have not revealed the relative expression of specific TRs across individual stages of hippocampal progenitor development. In this regard, the TRα1–GFP knockin mouse model (Wallis et al., 2010) provides an important tool to determine expression of this major TR isoform within adult hippocampal progenitors and in the hippocampal
neurogenic niche. Strikingly, studies with this reporter mouse line indicate a lack of GFP expression in proliferating (BrdU positive) hippocampal progenitors, with expression noted in postmitotic, progenitors committed to a neuronal fate (NeuroD-positive) (Kapoor et al., 2012). This then raises the intriguing possibility that TRα1 expression may only switch on once hippocampal progenitors exit the cell cycle. The current working model suggests that TRα1 expression switches on in postmitotic hippocampal progenitors and that TRα1 aporeceptor states would bias progenitors toward cell death, whereas liganded TRα1 activity would enhance cell survival and neuronal cell fate acquisition (Fig. 3). What remains unclear though is whether thyroid hormone through specific TR isoforms serves in anyway the role of an “intrinsic timer” determining the time point for cell cycle exit for hippocampal progenitors, as has been suggested for other progenitor cell types (Billon et al., 2002; Fernandez et al., 2004; Gao, Apperly, & Raff, 1998).

Although the data thus far have largely focussed on TRα1 receptors and their role in adult hippocampal progenitor development, TRβ isoforms are also expressed by hippocampal progenitors both in vivo and in vitro and are reported to regulate progenitor turnover (Desouza et al., 2005; Kapoor et al., 2012, 2011). TRβ−/− mice show increased proliferation of hippocampal progenitors as well as enhanced numbers of NeuroD-positive progenitor cells; however, this does not result in a consequent increase in DCX-positive immature neurons. These results are suggestive of a negative role of TRβ (either the apo- or holoreceptor form) in regulating hippocampal progenitor cell division. An increase in circulating levels of thyroid hormone in TRβ−/− mice (Forrest et al., 1996) may also contribute to neurogenic alterations observed in TRβ−/− mice. Also, the ability of TRβ isoforms to offset the mitogenic action of growth factors such as epidermal growth factors and IGF-1 in tumor cell lines (Martinez-Iglesias et al., 2009) opens the speculative possibility that the loss of TRβ may modify growth factor action on progenitors.

All of the above studies delineating the role of specific TR isoforms in hippocampal neurogenesis have employed mutants that are whole-body knockouts. These come with the inherent drawback of loss of function in embryonic and developmental stages as well, which may confound the effects observed on neurogenesis in adulthood. Further, since these animals are whole-body knockouts, they may exhibit alterations in T3, T4, and TSH levels, which could further confound the interpretation of the effects of TRs on adult neurogenesis (Flamant & Gauthier, 2013; O’Shea & Williams,
To overcome these drawbacks and gain a mechanistic understanding of TR contributions to different stages of neurogenesis, future studies require the development of conditional TR-specific knockout mice, which would allow spatiotemporal control over TR expression at specific stages of hippocampal progenitor development. Double mutants that disturb the stoichiometry of TR isoforms would also add deeper mechanistic insights to the interaction and function of the diverse TRs. Further, data from mutant mouse lines that disrupt deiodinase expression, as well as thyroid hormone transporter function, would provide a more complete picture of the effects of thyroid hormone on adult neurogenesis. To further elucidate the effects of thyroid hormone on different cell types of the neurogenic niche, studies in TR mutant mice are required that provide temporal and spatial control of TR expression in different subpopulations of the hippocampal niche namely astrocytes, mature granule cells, and microglia. These studies are needed to test the working idea that thyroid hormone may serve as a gating mechanism to promote cell cycle exit in dividing hippocampal progenitors, and the balance between TR aporeceptors and holoreceptors may then dynamically influence the numbers of progenitors that survive, undergo differentiation, and integrate into the hippocampal network.

4. THYROID HORMONE INFLUENCE ON ADULT SVZ NEUROGENESIS

4.1 Progression of SVZ Progenitor Development

Along the walls of the lateral ventricles lies the SVZ, which contains the largest number of dividing progenitors within the adult mammalian brain. The proliferative zone of the SVZ contains three types of progenitor cells (Fig. 1). The radial glia-like cells are the quiescent, multipotent neural stem cells or the type B cells. These divide asymmetrically to self-renew and give rise to the transit amplifying cells or type C cells, which in turn generate the neuroblasts or type A cells. The neuroblasts form a chain and migrate along the rostral migratory stream (RMS) to the olfactory bulb (OB), where they differentiate into different subtypes of interneurons—granule cells and periglomerular neurons, eventually integrating into existing OB neurocircuitry (Lim & Alvarez-Buylla, 2016; Ming & Song, 2011; Sakamoto, Kageyama, & Imayoshi, 2014). The proliferating type B cells express GFAP, Nestin, and Sox2. The type C cells express the homeobox transcription factor Dlx2 and the type A migrating neuroblasts are DCX and PSA-NCAM positive (Fig. 1) (Braun & Jessberger, 2014; Lim & Alvarez-Buylla, 2016). About
30,000 progenitors are produced in the SVZ everyday (Cameron & McKay, 2001; Ming & Song, 2005), and while many of these die, a reasonable fraction are slated to migrate to the OB and give rise to OB interneurons (Ming & Song, 2011; Zhao et al., 2008). Survival of SVZ progenitors is regulated at the neuroblast stage (Type A cells) and also at the time of immature neuron integration into OB circuitry (Lim & Alvarez-Buylla, 2016). SVZ neurogenesis is precisely regulated by both cell autonomous and noncell autonomous cues that adapt to alterations in the immediate local SVZ environment, as well as changes in the environment of the animal (Kuhn, Cooper-Kuhn, Eriksson, & Nilsson, 2005). The process of OB neurogenesis may contribute to the structural plasticity that is necessary for adaptations in olfactory discrimination, as newborn OB neurons have been reported to respond to novel odorant cues (Alvarez-Buylla & Garcia-Verdugo, 2002; Zhao et al., 2008). Among the major differences between SVZ and SGZ neurogenesis is that neuroblasts must migrate long distances tangentially and then radially to reach their final destination in the OB and develop into OB interneurons, while in the SGZ neuroblasts traverse a short trajectory within the DG subfield. It has only more recently been appreciated that adult neural stem cells both within the SVZ and SGZ not only exhibit clear differences between these neurogenic niches (Chaker, Codega, & Doetsch, 2016; Curtis, Low, & Faull, 2012), but also within any single niche neural stem cells are not homogeneous but rather exhibit heterogeneity at the level of proliferation potential, transcriptional expression, and diverse differentiation potential (Bonaguidi et al., 2016; Gebara et al., 2016; Giachino & Taylor, 2014; Goncalves et al., 2016; Jhaveri et al., 2015; Jhaveri, Taylor, & Bartlett, 2012; Llorens-Bobadilla et al., 2015).

### 4.2 Thyroid Hormone, TRs, and SVZ Neurogenesis

Distinct aspects of SVZ progenitor development have been reported to be sensitive to thyroid hormone fluctuations and regulated by specific TR isoforms (Fig. 4) (Lemkine et al., 2005; Lopez-Juarez et al., 2012). Adult-onset hypothyroidism results in a decline in SVZ progenitor proliferation with a failure noted in entry to cell cycle, resulting in an overall impairment of SVZ neurogenesis (Lemkine et al., 2005). Hypothyroid status is associated with a higher fraction of SVZ progenitors that continue to remain in interphase, resulting in an overall decline in proliferation. This reduction in SVZ neurogenesis and the decline in numbers of cycling progenitors are restored by exogenous thyroid hormone treatment. This has led to the view that T3...
may be important for exit from a quiescent stem cell-like state, and indeed, hypothyroid animals show a reduction in Dlx2 positive, rapidly amplifying Type C cells. In addition, adult-onset hypothyroidism is also associated with decreased apoptotic cell death in the SVZ and an overall decline in migratory neuroblasts in the RMS (Lemkine et al., 2005; Lopez-Juarez et al., 2012).

SVZ progenitor survival is also regulated by TTR, which is a choroid plexus-secreted protein that functions to regulate thyroid hormone active transport into the brain. TTR loss-of-function mice display lowered thyroid hormone levels in the brain, with a decrease observed in apoptotic cell loss and reduced cell death in the SVZ (Richardson, Lemkine, Alfama, Hassani, & Demeneix, 2007), phenocopying specific deficits in adult-onset hypothyroidism. In the influence on progenitor cell death, a very different phenotype is noted upon adult-onset hypothyroidism in the SVZ and SGZ with enhanced cell survival in the SVZ and increased cell death in the SGZ,

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**Fig. 4** Thyroid hormone regulation of adult SVZ neurogenesis. (A) Shown is a schematic of a coronal section through the adult rodent brain highlighting the neurogenic niche of the subventricular zone (SVZ) lining the lateral ventricles (LV). The schematic illustrates the developmental progression of adult SVZ progenitors from Type B quiescent neural progenitors to Type A migratory neuroblasts that travel along the rostral migratory stream to the olfactory bulb. (B) Shown is a table describing the effects of thyroid hormone on distinct stages of SVZ progenitor development, including studies from adult-onset perturbations of thyroid hormone levels and from TR isoform-specific mutant mouse models.
indicating that thyroid hormone exerts both distinct and overlapping effects in the regulation of progenitors in these two neurogenic niches. Taken together, these studies indicate that thyroid hormone influences diverse aspects of SVZ neurogenesis from regulating entry of quiescent stem cells into the cell cycle, cell death within proliferating progenitors in the niche and acquisition of a neuronal cell fate (Fig. 4).

Thus far, reports indicate that the sole TR isoform expressed in the SVZ is TRα1 (Lemkine et al., 2005; Lopez-Juarez et al., 2012), with reports indicating that TRα1 is not present in the quiescent neural stem cells, but rather appears in the Dlx2-positive, rapidly amplifying type C cells and is present at high levels in the DCX-positive migratory neuroblast (Lopez-Juarez et al., 2012). The absence of TRα1 in type B progenitor pool, and its expression in the type C and type A cells, suggests the possibility that TRα1 may drive progenitors to differentiate and commit to a neuronal fate. As a corollary to this, TRα1 overexpression was found to increase the number of neuroblast cells (Fig. 4) (Lopez-Juarez et al., 2012). In contrast, siRNA knockdown of TRα1 resulted in an increase in the type B progenitor pool, thought to arise due to a loss of repressive effects of thyroid hormone/ TRα1 on the expression of Sox2, which causes progenitor cells to retain their stem cell identity and prevents differentiation (Lopez-Juarez et al., 2012). This is also phenocopied in the TRα1/0 mice, wherein the progenitors do not progress toward neuronal commitment, leading to an accumulation of quiescent, nonproliferating progenitors (Lemkine et al., 2005; Lopez-Juarez et al., 2012). The prevailing view is that the liganded TRα1 receptor enhances SVZ progenitor maturation toward a neuronal lineage through the repression of stem cell identity genes like Sox2 and also the repression of cell cycle genes such as cyclinD1 and c-myc (Lemkine et al., 2005; Lopez-Juarez et al., 2012), causing proliferating progenitors to exit cell cycle and progress toward a neuroblast identity (Fig. 5). This is similar to the pattern of TRα1 expression in the SGZ progenitors within the hippocampal neurogenic niche, suggesting a certain common theme between both of these adult progenitors wherein TRα1 may play the role of a gatekeeper during progenitor development promoting acquisition of a neuronal fate.

Studies on SVZ progenitor cells in vitro have demonstrated that when neural stem cells are cultured from hyperthyroid animals, they show increased oligodendrocyte differentiation (Fernandez et al., 2004). This suggests that much like SGZ progenitors, SVZ progenitors when removed from the neurogenic niche seem to respond to thyroid hormone quite differently. Both SGZ and SVZ progenitors show a greater propensity for glial fates when treated with thyroid hormone in vitro (Desouza et al., 2005;
Fernandez et al., 2004), whereas in vivo studies (Kapoor et al., 2012, 2010; Lemkine et al., 2005; Lopez-Juarez et al., 2012) indicate enhanced neuronal differentiation of both SVZ and SGZ progenitors in response to thyroid hormone. This opens up the possibility of distinct effects on progenitors while...
present within their neurogenic niches and when in isolated conditions, thus motivating experiments to address the effects of thyroid hormone on diverse aspects of the neurogenic niche. Further, there are no reports investigating the presence of deiodinases or thyroid hormone transporters in the SVZ. Given the key role that astrocytes within the SVZ niche have been reported to play, it is important to study how local thyroid hormone levels may be dynamically influenced via D2/D3 activity balance within diverse cell types of the SVZ neurogenic niche. Studies on TR isoforms thus far have focussed predominantly on the TR\(\alpha_1\) isoform, and while other isoforms have not been reported in the SVZ so far, a systematic analysis of their expression merits investigation. Additionally, mutant mouse lines that afford spatial and temporal control of TR\(\alpha_1\) expression, within specific stages of SVZ progenitor development, would help to elucidate the effects of thyroid hormone at each stage. This would also enable the delineation of thyroid hormone target genes at individual stages of progenitor development.

5. UNDERLYING MOLECULAR MEDIATORS FOR THE REGULATION OF NEUROGENESIS BY THYROID HORMONE

At the nuclear level, thyroid hormone mediates its effects via TRs, which serve as transcription factors at TREs driving the regulation of thyroid hormone target genes. TRs toggle between an unliganded aporeceptor state and a ligand–bound state, regulating gene transcription differentially in both forms (Bernal et al., 2015; Brent, 2012; Chassande, 2003; Cheng et al., 2010; Harvey & Williams, 2002). The TRs bound to TREs at thyroid hormone target genes exist in complexes with coactivators or corepressors, switching on or off gene transcription (Bernal & Morte, 2013; Bianco, 2011; Bianco & Kim, 2006; Lee & Privalsky, 2005; Schroeder & Privalsky, 2014). Depending on the nature of the TRE, they can either activate or repress transcription. Positive TREs are more common, where TR aporeceptors repress transcription by complexing with corepressors that possess histone deacetylase activity (Chassande, 2003; Zhang & Lazar, 2000). The repression by TR aporeceptors is lifted by thyroid hormone binding to the aporeceptor in conjunction with recruitment of coactivators that exhibit histone acetylase activity and mediate recruitment of RNA polymerase II, thereby initiating transcription (Aстапова & Hollenberg, 2013; Cheng et al., 2010; Harvey & Williams, 2002; Liu & Brent, 2010). On the other hand, negative TREs are those wherein aporeceptor activity leads to switching on of target
genes and ligand binding serves to repress gene transcription ([Wu & Koenig, 2000]). The coactivators ([Goodman & Smolik, 2000; Ito & Roeder, 2001]) or corepressors ([Aastapova & Hollenberg, 2013; Hu & Lazar, 2000; Privalsky, 2004]) that communicate with transcriptional machinery are part of multi-subunit complexes with enzyme activities. Over and above histone acetylation/deacetylation activities, these complexes can also have enzymes such as methylases, kinases, or phosphatases which act in concert with TR isoforms, to fine tune gene expression ([Bernal & Morte, 2013; Cheng et al., 2010]). Thyroid hormone also exerts nongenomic actions through modulation of membrane receptors and signaling pathways ([Bitiktas et al., 2016; Davis et al., 2010; Lanni et al., 2016]).

While several studies have reported thyroid hormone target genes ([Chatonnet, Flamant, & Morte, 2015]) during neurodevelopment and in neuronal cells, including Reelin ([Alvarez-Dolado et al., 1999]), Stat3 ([Chen et al., 2012]), Tag1 ([Alvarez-Dolado et al., 2001]), Sox2, Jun, Notch1, Notch4, Klf7, Ngf, Pax9, Slit2 ([Chatonnet, Guyot, Benoit, & Flamant, 2013]), and Dab ([Alvarez-Dolado et al., 1999]), thus far only a few bona fide target genes have been identified in adult progenitors. At present, there is a paucity of understanding of the molecular mechanisms that mediate the neurogenic actions of thyroid hormone within the adult mammalian brain. In the SVZ, the current model proposes that TRα1 aporeceptor activity at negative TREs activates the expression of genes that regulate stem cell identity, as well as genes regulating cell cycle entry to maintain cells in a proliferating undifferentiated state ([Fig. 5]) ([Lemkine et al., 2005; Lopez-Juarez et al., 2012]). Thyroid hormone binding to its aporeceptor would cause repression of these genes that likely contain negative TREs, leading to cell cycle exit and differentiation to neuroblasts ([Fig. 5]). In this context, the stem cell–associated gene Sox2 and the cell cycle genes (cyclin D1 and c-myc) have been identified as thyroid hormone target genes that are repressed in response to thyroid hormone in neural stem cells of the SVZ ([Lemkine et al., 2005; Lopez-Juarez et al., 2012]). Thyroid hormone repression of cell cycle regulatory genes and genes associated with “stemness” through TRα1 raises the possibility that thyroid hormone may exert a permissive role on SVZ neural stem cells enhancing cell cycle exit and acquisition of a differentiated phenotype. What is unclear at present is whether thyroid hormone also contributes to the activation of specific proneural genes in SVZ stem cells, thus also actively promoting the acquisition of a neuronal cell fate. It is likely that Sox2, c-myc, and cyclin D1 represent only a subset of the thyroid hormone responsive genes. A more detailed understanding of the
transcriptional targets for thyroid hormone in SVZ progenitors across their developmental progression is required to determine whether thyroid hormone plays a more passive permissive role in this niche, or whether it actively targets gene programs to drive neuronal cell fate determination.

In the hippocampal SGZ, the current model proposes that TRα1 expression may switch on as neural stem cells exit cell cycle. TRα1 aporeceptor activity in postmitotic hippocampal progenitors would then result in repression of prosurvival genes and eventual cell death in the absence of thyroid hormone (Fig. 3). In contrast, TRα1 holoreceptor would activate gene expression of both prosurvival and proneural genes, enhancing progenitor survival and differentiation into neurons (Fig. 3). While thus far no bona fide thyroid hormone target genes have been demonstrated in hippocampal progenitors, both in vivo and in vitro studies have highlighted putative gene targets. In response to thyroid hormone administration in vivo, the transcripts of multiple genes associated with neuronal progenitor development such as Tis21, Tlx, Dlx2, Math-1, and Ngn1 were robustly upregulated (Kapoor et al., 2012). Of these, activation of Tis 21, which is expressed in Type 2 and 3 progenitors, is known to hasten neuronal differentiation of progenitors (Attardo et al., 2010; Farioli-Vecchioli et al., 2009), akin to that seen following thyroid hormone treatment. Interestingly, treatment of hippocampal derived neurospheres by thyroid hormone enhanced expression of proneural genes Emx2 and Klf9, indicating a distinct pattern of regulation from in vivo studies (Kapoor et al., 2012). It is important to note that at present whether any of these genes serve as genuine thyroid hormone target genes with recruitment of specific TRs to TRE sequences in their promoters remains to be further ascertained.

Chromatin immunoprecipitation sequencing (ChIP-seq) data using TR isoform–specific antibodies is essential to elucidate the specific transcriptional targets of thyroid hormone across the genome at different stages of SGZ and SVZ progenitor development. Currently, these studies have been hindered by the nonavailability of TR isoform–specific ChIP grade antibodies. Nonetheless, studies have taken advantage of ChAP experiments with GST–tagged TR isoforms or pan-RXR ChIPs (Chatonnet et al., 2013) or made use of available GFP-labeled TR isoform–specific knockin mice (Dudazy-Gralla et al., 2013), to investigate TR–specific target genes in neural cells. These nature of experiments open up the possibility of gaining important understanding of the sites where TRs bind within the genome of SVZ and SGZ progenitors, and the manner of regulation mediated by thyroid hormone at target genes through both negative and positive
TREs. Further, the nongenomic actions of thyroid hormone within neurogenic niches and in adult neuronal progenitors also warrant further investigation. In toto, the molecular effects that arise downstream of thyroid hormone in the SGZ and SVZ progenitor pool remain poorly delineated and need further investigation to determine the mechanistic underpinnings of the effects of thyroid hormone on regulation of neural stem cell quiescence, cell cycle entry and exit, progenitor survival, cell fate acquisition, progenitor maturation, and integration into neuronal networks.

6. FUNCTIONAL IMPLICATIONS OF THE NEUROGENIC EFFECTS OF THYROID HORMONE

6.1 Thyroid Hormone Regulation of Behavior in Animal Models

The behavioral implications of thyroid hormone-mediated regulation of adult hippocampal and OB neurogenesis remain poorly understood. Studies in rodent models implicate adult hippocampal neurogenesis in the regulation of hippocampal dependent learning and memory (Deng, Aimone, & Gage, 2010; Lieberwirth et al., 2016; Stuchlik, 2014; Yau, Li, & So, 2015) as well as in the regulation of anxiety and mood behavior (Cameron & Glover, 2015; Hage & Azar, 2012; Miller & Hen, 2014). In this regard, adult-onset hypothyroidism evokes significant impairments in learning tasks (Fundaro, 1989) and also enhances behavioral despair on tasks such as the forced swim test considered to provide a measure for depressive-like behavior (Kulikov, Torresani, & Jeanningros, 1997; Montero-Pedrazuela et al., 2006). These correlative observations suggest that the decline in hippocampal neurogenesis noted in adult-onset hypothyroid animals may contribute to the cognitive and mood-related behavioral changes observed in these animals. Interestingly, TR mutant mice, such as the dominant-negative TRα1+/m mice that have a significant increase in TRα1 aporeceptor activity, show enhanced anxiety behavior and also have performance deficits in recognition memory tasks (Pilhatsch et al., 2010; Venero et al., 2005). This behavioral phenotype in TRα1+/m mice is associated with a significant reduction in ongoing adult hippocampal neurogenesis (Kapoor et al., 2010). These studies highlight that in an unliganded state TR isoforms, in particular TRα1, evoke cellular changes of a neurogenic decline in the hippocampus accompanied by impaired cognition and perturbed mood behavior. Though at present these links remain correlative, given the building evidence linking hippocampal neurogenesis to the modulation of learning, memory, and
mood (Cameron & Glover, 2015; Hage & Azar, 2012; Lieberwirth et al., 2016; Miller & Hen, 2014; Yau et al., 2015), it is tempting to speculate that there may exist causal links that warrant further investigation. SVZ neurogenesis has been linked to olfactory perception, discrimination learning and memory (Hoyk, Szilagyi, & Halasz, 1996; Mackay-Sim & Beard, 1987; Malvaut & Saghatel, 2016; Paternostro & Meisami, 1991, 1996; Sakamoto et al., 2014). Rodent models of adult-onset hypothyroidism exhibit a reduction in their olfactory perception (Beard & Mackay-Sim, 1987; Paternostro & Meisami, 1993, 1996); conversely hyperthyroid animals exhibit increased olfactory responsiveness (Brunjes, Schwark, & Greenough, 1982; Johanson, 1980). Although at present it is a leap to conclude that neurogenic changes brought about in the OB due to perturbations of SVZ neurogenesis contribute to the effects of thyroid hormone on olfaction, this notion requires experimental investigation to identify any possible link. The understanding of the contributions of the neurogenic effects to thyroid hormone influenced behavioral consequences remains very limited and largely correlative.

6.2 Clinical Relevance of the Neurogenic Actions of Thyroid Hormone

Clinical thyropathies span diverse conditions including overt hypo- and hyperthyroidism and their subclinical counterparts, Hashimoto’s thyroiditis, thyrotoxicosis associated with Graves’ disease, thyroid goiters, benign thyroid nodules, and thyroid cancers (Cooper & Biondi, 2012; Franklyn & Boelaert, 2012; Jones, May, & Geraci, 2010). Furthermore, they also include “nonthyroidal illness syndrome” and resistance to thyroid hormone—a syndrome with reduced thyroid hormone responsiveness (Cooper & Biondi, 2012; Olateju & Vanderpump, 2006). Hypothyroidism is one of the most prevalent endocrine disorders with a global incidence of 5%–9% in the general population and is particularly prevalent in menopausal women (Canaris, Manowitz, Mayor, & Ridgway, 2000; Stuenkel, 2015; Unnikrishnan & Menon, 2011). In addition to the pathophysiological conditions described earlier, it is also possible that more subtle changes to thyroid hormone-mediated actions could arise through disruption of normal thyroid hormone signaling via perturbations of local deiodinase activity within specific brain regions, perturbation of TR isoforms, and their relative stoichiometry giving rise to subclinical situations wherein thyroid hormone function is hampered.

Adult-onset thyroid disorders, both hypothyroidism and hyperthyroidism, have long been recognized to be associated with cognitive impairments,
anxiety, and mood instability (Fig. 6) (Bathla, Singh, & Relan, 2016; Bauer et al., 2008; Berent, Zboralski, Orzechowska, & Galecki, 2014; Demartini et al., 2014; Dugbartey, 1998; Ittermann, Volzke, Baumeister, Appel, & Grabe, 2015; Jackson, 1998). Though overt hypothyroidism can adversely affect diverse domains of cognition (Capet et al., 2000; Smith, Evans, Costall, & Smythe, 2002), among the most sensitive is memory retrieval, in particular for verbal memory (Gobel et al., 2016; Miller et al., 2007). Further, a cross-sectional and interventional study in subclinical hypothyroid patients with elevated TSH but normal free T4 indicated mild cognitive impairments in hippocampal dependent memory tasks, which were reversed upon hormone replacement. In contrast, the cognitive impairments in overtly hypothyroid patients did not show a complete reversal, highlighting

**Fig. 6** Behavioral implications of thyroid hormone action on adult neurogenesis. Shown is a sagittal schematic view of the human brain, depicting the neurogenic niches of the hippocampus and olfactory bulb, and also the hypothalamic–pituitary–thyroid axis that regulates thyroid hormone secretion from the thyroid gland. Clinical evidence links thyroid hormone dysfunction to perturbations in cognition, mood, and anxiety, as well as in the sensory modality of olfaction. Studies in preclinical models indicate that thyroid hormone regulates both hippocampal and olfactory bulb neurogenesis, highlighting the importance of studies focused on examining the behavioral significance of the neurogenic actions of thyroid hormone.
the importance of early detection and treatment for full recovery of cognitive symptoms (Correia et al., 2009). Hippocampal volumetric analysis based on MRI studies indicated volumetric loss associated with adult-onset hypothyroidism in human patients (Cooke, Mullally, Correia, O’Mara, & Gibney, 2014). An interesting imaging study of mildly hypothyroid patients indicated a negative correlation of hippocampal volume with serum levels of TSH (Daghighi et al., 2016), suggesting that potential volumetric changes may arise even prior to overt hypothyroidism onset. It would be of interest to address whether such changes in hippocampal volume can be restored following hormone replacement therapy. Although animal studies highlight the neurogenic actions of thyroid hormone, clinical data either on human neural stem cells or through postmortem analysis are lacking. Such studies would address whether the effects of thyroid hormone on neural stem cells observed in animal models crossover to studies with humans.

Thyroid hormone dysfunction is also linked to affective disorders, with enhanced susceptibility to depressive symptomatology in hypothyroid patients and increased anxiety noted in hyperthyroid subjects (Dayan & Panicker, 2013; Hage & Azar, 2012; Kathol & Delahunt, 1986). Further, thyroid hormone is used as an effective adjunct treatment to pharmacological antidepressants and has been suggested to accelerate and augment their therapeutic efficacy (Bauer & Whybrow, 2001; Cooper-Kazaz et al., 2007; Haggerty & Prange, 1995; Henley & Koehnle, 1997; Uhl et al., 2014). Given clinical evidence supports a hippocampal volumetric decline in patients of major depression (Bremner et al., 2000; Videbech & Ravnkilde, 2004), imaging studies that address the importance of thyroid hormone augmentation strategies in being able to reverse such structural correlates are of interest. Preclinical studies highlight that pharmacological antidepressants (Malberg, Eisch, Nestler, & Duman, 2000) and thyroid hormone (Kapoor et al., 2015) both enhance adult hippocampal neurogenesis, albeit influencing distinct stages of hippocampal progenitor development. While antidepressants enhance turnover and morphological maturation of hippocampal progenitors (Madsen, Yeh, Valentine, & Duman, 2005; Perera et al., 2007; Santarelli et al., 2003), thyroid hormone enhances postmitotic survival and neuronal differentiation (Kapoor et al., 2012, 2010). This then suggests that in combination, these treatments may work to significantly increase diverse aspects of hippocampal neurogenesis and indeed this is borne out in preclinical studies (Eitan et al., 2010). However, there is a paucity of information from clinical studies, imaging observations,
or using human neural stem cells that directly examines the importance of the neurogenic effects of thyroid hormone in aiding specific actions of antidepressant therapies.

While clinical evidence has provided more support for altered hippocampal mediated behaviors in patients with thyroid hormone dysfunction, nevertheless, thyroid hormone dysfunction has also been linked to the regulation of smell (Fig. 6). Hypothyroid patients are reported to exhibit dysosmia, which can be restored by hormone replacement (Deniz et al., 2016; Gunbey et al., 2015). While the olfactory impairments are thought to arise due to effects of thyroid hormone on olfactory receptor neurons in the olfactory epithelium (Paternostro & Meisami, 1993, 1996), given the preclinical studies linking thyroid hormone to regulation of OB neurogenesis, it seems premature to preclude a role for the neurogenic effects of thyroid hormone. In general, the extent of ongoing neurogenesis is thought to be highly restricted in the human brain with evidence, thus far supporting progenitor turnover in the human hippocampus (Eriksson et al., 1998; Spalding et al., 2013), but limited for the SVZ (Ernst & Frisen, 2015; van Strien, van den Berge, & Hol, 2011). This factor of course is important to bear in mind when extrapolating the findings of the neurogenic effects of thyroid hormone from animal models to humans. However, this only serves to highlight the lacunae and the importance of studies that address the effects of thyroid hormone on neural stem cells of human origin.

7. CONCLUSION

Thyroid hormone exerts a key instructive influence on adult hippocampal and SVZ progenitors, regulating their exit from cell cycle, survival, and commitment to a neuronal fate. These actions are mediated via TR isoforms, which in their unliganded vs liganded state can evoke differing consequences for the developmental trajectory of adult progenitors. It is critical to study the functional and behavioral consequences of these neurogenic effects of thyroid hormone, expanding preclinical studies to the use of human neural stem cells to address the relevance of findings in animal models. Thyroid hormone dysfunction is among the most prevalent of endocrine disorders, linked to both cognitive and psychiatric symptoms, highlighting the importance of studies that aid in the development of a deep mechanistic understanding of the actions of thyroid hormone.
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