Unliganded thyroid hormone receptor α1 impairs adult hippocampal neurogenesis

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ABSTRACT **Thyroid hormone regulates adult hippocampal neurogenesis, a process involved in key functions, such as learning, memory, and mood regulation. We addressed the role of thyroid hormone receptor TRα1 in adult hippocampal neurogenesis, using mice harboring a TRα1 null allele ($TR\alpha1^{-/-}$), overexpressing TRα1 6-fold ($TR\alpha2^{-/-}$), and a mutant TRα1 ($TR\alpha1^{+/m}$) with a 10-fold lower affinity to the ligand. While hippocampal progenitor proliferation was unaltered, $TR\alpha1^{-/-}$ mice exhibited a significant increase in doublecortin-positive immature neurons and increased survival of bromodeoxyuridine-positive ($BrdU^+$) progenitors as compared to wild-type controls. In contrast, the $TR\alpha1^{+/m}$ and the $TR\alpha2^{-/-}$ mice, where the overexpressed TRα1 acts as an aporeceptor, showed a significant decline in surviving $BrdU^+$ progenitors. $TR\alpha1^{-/-}$ and $TR\alpha2^{-/-}$ mice showed opposing effects on neurogenic markers like polysialylated neural cell adhesion molecule and stathmin. The decreased progenitor survival in the $TR\alpha2^{-/-}$ and $TR\alpha1^{+/m}$ mice could be rescued by thyroid hormone treatment, as was the decline in neuronal differentiation seen in the $TR\alpha1^{+/m}$ mice. These mice also exhibited a decrease in $NeuroD^+$ cell numbers in the dentate gyrus, suggesting an effect on early postmitotic progenitors. Our results provide the first evidence of a role for unliganded TRα1 in modulating the deleterious effects of hypothyroidism on adult hippocampal neurogenesis.—Kapoor, R., van Hogerlinden, M., Wallis, K., Ghosh, H., Nordstrom, K., Vennstrom, B., Vaidya, V. A. Unliganded thyroid hormone receptor α1 impairs adult hippocampal neurogenesis. *FASEB J.* 24, 4793–4805 (2010). www.fasebj.org**

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THYROID HORMONE PERTURBATIONS in development cause major neuroanatomical and neurological deficits (1). Though adult-onset hypothyroidism does not manifest as severely as developmental hypothyroidism (2), it can precipitate depressive behavior and deteriorate cognitive function (3, 4). In particular, adult hypothyroidism impairs hippocampus-dependent behaviors, resulting in learning, memory, and mood-related deficits (5, 6). Adult hippocampal neurogenesis plays an important role in these hippocampus-dependent tasks (7, 8), and is regulated by thyroid hormone (9–11). Decreased hippocampal neurogenesis has been postulated to contribute to the deficits in hippocampal functions observed in adult-onset hypothyroidism.

Adult neurogenesis encompasses progenitor proliferation, survival and differentiation, and the maturation and functional integration of newborn neurons (12). The developmental stages of adult neurogenesis are characterized by stage-specific markers, such as nestin, NeuroD, doublecortin (DCX), polysialylated neural cell adhesion molecule (PSA-NCAM), stathmin, and calretinin (13, 14). Adult hypothyroidism decreases progenitor survival, DCX-positive (DCX^+) immature neuron number, and neuronal differentiation, with no effect on progenitor proliferation (9, 10). *In vitro* evidence suggests a direct effect of thyroid hormone on adult hippocampal progenitors (10). However, the role of thyroid hormone receptors (TRs) and their contribution to the damaging effects of hypothyroidism on adult hippocampal neurogenesis is unknown.

TRs are transcription factors that bind thyroid hormone response elements and activate or repress target genes as ligand-receptor complexes or aporeceptors (15). TR α and β genes generate several TR isoforms, of which TRα1, TRα2, TRβ1, and TRβ2 are predominant in the adult mammalian brain (2). TRα1 contributes 70–80% of total TR expression in the brain (16). TRα2 does not bind thyroid hormone, though some reports implicate TRα2 in the transcriptional repression of thyroid hormone-responsive genes (17). It is of interest to note that the phenotype in $TR\alpha2^{-/-}$ mice, which as a consequence of ablation of TRα2 inevitably overex-

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press TRα1 severalfold, has been ascribed to TRα1 aporeceptor effects in many tissues (18).

It remains unclear whether the deleterious effects of hypothyroidism are due to insufficient target gene activation or a consequence of the aporeceptor acting as a transcriptional regulator (15). The focus of the present study was to investigate the role of TRα1 in adult hippocampal neurogenesis, using TRα1$^{-/-}$, TRα2$^{-/-}$, and TRα1$^{+/m}$ heterozygous mice carrying a point mutation (TRα1R384C) that lowers thyroid hormone affinity 10-fold (18–20). Further, TRα1-GFP-expressing mice were used to address the stage-specific expression of TRα1 in adult hippocampal progenitors. Our results demonstrate a key role for TRα1 in the regulation of the postmitotic survival of adult hippocampal progenitors, and indicate that an unliganded TRα1, acting as an aporeceptor, is responsible for the deleterious effects of hypothyroidism on adult hippocampal neurogenesis.

MATERIALS AND METHODS

Thyroid hormone receptor mutant mice

TRα1$^{-/-}$ and TRα2$^{-/-}$ mice were generated as described previously (18, 19). The mouse strain carrying the dominant-negative R384C mutation in TRα1 (TRα1$^{+/m}$) was generated as described previously (20). TRα1-GFP-knock-in mice were constructed by inserting the coding sequence of eGFP in frame 3′ to exon 9 of the TRα1 gene (21). Heterozygote offspring were bred against C57BL/6 for 3 generations and then intercrossed to generate TRα1-GFP mice homozygous for the chimeric gene. TRα1-GFP-knock-in mice have normal body and organ weights and T3, T4, and TSH levels, and show no overt morphological or physiological phenotype. The littermate mutant and wild-type mice were kept at 21°C on a 12:12-h light-dark cycle, and 2-mo-old male mice were used in the study. Animal care procedures were in accordance with the guidelines set by the European Community Council Directives (86/609/EEC) and were approved by the Karolinska Institutet and Tata Institute of Fundamental Research (TIFR) institutional animal ethics committees.

BrdU-labeling paradigms and drug treatments

To determine whether TRα1 is expressed by proliferating adult hippocampal progenitors, we injected heterozygote TRα1-GFP mice with a single intraperitoneal (i.p.) injection of the mitotic marker 5-bromo-2′-deoxyuridine [BrdU, 150 mg/kg body weight (bw); Sigma, St. Louis, MO, USA], 2 h prior to sacrifice (n=3). Male wild-type mice were used as a negative control to ensure specificity of the GFP signal. To address the effects on adult hippocampal progenitor proliferation, TRα1$^{-/-}$ and TRα2$^{-/-}$ mice, as well as littermate wild-type controls, received a single i.p. injection of BrdU (100 mg/kg bw; Sigma) and were sacrificed 2 h later (n=4 or 5/group). To address the role of an unliganded TRα1 receptor on adult hippocampal progenitor proliferation, TRα1$^{+/m}$ and wild-type littermate controls received a single BrdU (100 mg/kg bw) injection and were sacrificed 2 h later (n=4–5/group).

To examine effects on the survival and differentiation of adult hippocampal progenitors, TRα1$^{-/-}$ and TRα2$^{-/-}$ mice, as well as littermate wild-type controls were administered BrdU (150 mg/kg bw) once daily by i.p. injection for 3 d and were sacrificed 28 d after the last injection (n=4 or 5/group). To examine the role of an unliganded TRα1 receptor on adult hippocampal progenitor survival and differentiation, TRα1$^{+/m}$ and wild-type littermate controls received a single daily BrdU (100 mg/kg bw) injection for 3 d and were sacrificed 30 d later (n=4 or 5/group).

Experiments to address the influence of thyroid hormone treatment on adult hippocampal progenitor survival in TRα2$^{-/-}$ mice had 4 experimental groups: wild type + vehicle, wild type + T3, TRα2$^{-/-}$ + vehicle, and TRα2$^{-/-}$ + T3 (n=4 or 5/group). The groups receiving thyroid hormone treatment were given T3 (0.5 μg/ml, Sigma) in drinking water, as described previously (22) for 16 d prior to sacrifice.

In experiments to examine the effect of thyroid hormone treatment on adult hippocampal progenitor survival and differentiation in TRα1$^{+/m}$ mice, there were 4 experimental groups: wild type + vehicle, wild type + T3, TRα1$^{+/m}$ + vehicle, and TRα1$^{+/m}$ + T3 (n=4–5/group). The treatment groups (wild type + T3, TRα1$^{+/m}$ + T3) received T3 (0.5 μg/ml) in drinking water, as described previously (22) for 12 d. From d 10 to d 12, all animals were administered a single injection of BrdU (150 mg/kg bw), and were sacrificed 30 d after the last BrdU injection.

Immunohistochemistry

Mice were sacrificed by transcardial perfusion with 4% PFA, brains were removed, postfixed, and subsequently cryoprotected in 30% sucrose-PFA. Serial coronal sections (30 μm) through the rostrocaudal extent of the hippocampus were generated using a freezing microtome (Leica, Wetzlar, Germany). Free-floating sections were processed for BrdU immunohistochemistry as described previously (10). In brief, after DNA denaturation and acid hydrolysis, sections were incubated overnight with mouse anti-BrdU antibody (1:500; Boehringer Mannheim, Indianapolis, IN, USA). Sections were then exposed to secondary antibody (biotinylated anti-mouse IgG, 1:500, Vector Laboratories, Burlingame, CA, USA). An avidin-biotin complex (Vector Laboratories) was used for signal amplification, which was detected using the substrate diaminobenzidine (Sigma).

For immunohistochemistry and immunofluorescence experiments to examine the expression of endogenous markers of either immature neurons (DCX, PSA-NCAM) or the neurogenic niche (NeuroD, stathmin) in the adult hippocampus, tissue sections were blocked using 10% horse serum in phosphate buffer prior to overnight exposure at room temperature to the following antibodies: goat anti-DCX (1:250, Santa Cruz Biotechnology, Santa Cruz, CA, USA), mouse anti-PSA-NCAM (1:500; kind gift from Prof. T. Seki, Juntendo University, Tokyo, Japan; ref. 23), rabbit anti-stathmin (1:250; Calbiochem, San Diego, CA, USA), and goat anti-NeuroD (1:200; Santa Cruz Biotechnology). Following washes in 0.1 M phosphate buffer, sections were incubated with secondary antibodies: biotinylated anti-goat IgG (1:250; Vector Laboratories), Alexa 488-conjugated donkey anti-mouse (1:250; Molecular Probes, Eugene, OR, USA), Alexa 488-conjugated donkey anti-rabbit (1:250, Jackson ImmunoResearch, Bar Harbor, ME, USA) at room temperature for 3 h. An avidin-biotin complex (Vector Laboratories) was used for signal amplification of biotinylated secondary antibodies, which was then detected with diaminobenzidine (Sigma). To visualize immunofluorescence, sections were mounted in Vectashield (Vector Laboratories) and viewed using a Nikon Eclipse 90i fluorescence microscope (Nikon, Tokyo, Japan).

For experiments to address the neuronal differentiation of adult dentate granule cell progenitors, double-labeling experiments were carried out. Sections were incubated overnight

with the following primary antibody cocktails: rat anti-BrdU (1:500; Accurate Biochemicals, Westbury, NY, USA) with mouse anti-neuronal nuclei (NeuN) (1:1000; Chemicon, Temecula, CA, USA) to assess the neuronal differentiation of adult hippocampal progenitors; or rat anti-BrdU (1:500; Covance, Vienna, VA, USA) with rabbit anti-calretinin (1:250; Swant, Bellinzona, Switzerland) to examine the colocalization of BrdU with the transient immature neuron marker, calretinin. Sections were then incubated with the following cocktails of secondary antibodies: biotinylated anti-rat IgG (1:500; Chemicon) with Alexa 555-conjugated anti-mouse (1:250; Molecular Probes) or Cy3-conjugated donkey anti-rat and FITC-conjugated donkey anti-mouse (both 1:250; Jackson ImmunoResearch). Signal amplification was performed using FITC-conjugated streptavidin (1:250; Molecular Probes), following which sections were mounted using Vectashield (Vector Laboratories). Sections were observed with a Zeiss Axiovert confocal laser scanning microscope (510LSM; Carl Zeiss, Oberkochen, Germany) to determine immunofluorescence colocalization using confocal *z*-plane sectioning.

For experiments to determine the neuronal or glial differentiation of adult dentate granule cell progenitors, triple-labeling studies were carried out. Sections were incubated overnight with a cocktail of primary antibodies: rat anti-BrdU (1:500; Accurate Biochemicals) with mouse anti-NeuN (1:1000; Chemicon) and rabbit anti-glial fibrillary acidic protein (GFAP; 1:500; Chemicon). Sections were incubated with a cocktail of secondary antibodies: biotinylated anti-rat IgG (1:500; Chemicon) with Alexa 555-conjugated anti-mouse (1:250; Molecular Probes) and Cy5-conjugated anti-rabbit (1:500; Chemicon), followed by incubation with Alexa 488-conjugated streptavidin (1:500, Molecular Probes). Sections were mounted using Vectashield (Vector Laboratories), and immunofluorescence colocalization was determined using confocal *z*-plane sectioning with an Olympus Fluoview FV 1000 laser scanning microscope (Olympus, Tokyo, Japan).

For double labeling in TRα1-GFP mice, sections were incubated overnight with rabbit anti-GFP (1:5000; Abcam, Cambridge, UK) along with either goat anti-NeuroD (1:200) or goat anti-DCX (1:250; both Santa Cruz Biotechnology), followed by a cocktail of secondary antibodies: anti-goat Alexa 594 and anti-rabbit Alexa 488 (both 1:1000; Invitrogen, Stockholm, Sweden). For BrdU double-labeling, the sections were first incubated with anti-GFP primary and secondary antibodies before BrdU pretreatment and immunohistochemistry, as described above. The GFP signal was specific as demonstrated by the lack of any immunofluorescence with an anti-GFP antibody in wild-type mice. Colocalization of GFP signal in TRα1-GFP mice with the markers (BrdU, NeuroD, and DCX) was examined using confocal *z*-stack images on a Zeiss Axiovert confocal laser-scanning microscope (510LSM). For all immunohistochemistry and immunofluorescence experiments, the specificity of antibody signal was confirmed using the following controls: absence of primary antibody, or isotype-matched IgG controls.

Cell counting

Quantitation of $BrdU^+$ cell numbers in hippocampal sections was carried out using a previously described modified, unbiased stereology protocol (24) on a Zeiss Axioskop microscope. Sections spanned the rostrocaudal extent of the hippocampus (bregma −1.34 to −3.80) (25), and every 6th hippocampal section was processed for quantitation (12 sections/animal). Sections were coded, and the quantitation was conducted by an experimenter masked to the code. $BrdU^+$ cells within dentate gyrus (DG) were counted as being in the subgranular zone (SGZ)/granule cell layer (GCL) when they were directly touching the SGZ or within it. The total number of $BrdU^+$ cells in the SGZ/GCL was estimated by multiplying the total number of BrdU cells counted from every 6th section by the section periodicity, 6.

Quantitation of DCX^+ cells in hippocampal sections was carried out by an experimenter masked to the study code. The number of DCX^+ cells in the SGZ of the DG were quantitated (4 sections/animal, n=5/group). The results were expressed as the number of DCX^+ cells per section. We also addressed the morphological status of DCX^+ cells by categorizing them as DCX^+ cells without tertiary dendrites or DCX^+ cells with complex tertiary arbors (26). Quantitation of both DCX^+ cell numbers and morphological category was performed under masked conditions using a Zeiss Axioskop at ×400.

Quantitation of proliferating cell nuclear antigen (PCNA), PSA-NCAM, stathmin, and $NeuroD^+$ cells was performed using the same approach used for quantitation of DCX^+ cell numbers.

To examine the differentiation of $BrdU^+$ progenitors into neurons or glia in the $TR\alpha1^{-/-}$ and $TR\alpha2^{-/-}$ mice, the percentage of $BrdU^+$ cells that colocalized with the neuronal marker NeuN or the glial marker GFAP was determined using confocal microscopy. In each animal (n=5/group), 20 $BrdU^+$ cells were analyzed using *z*-plane sectioning with 0.41-μm steps on an Olympus Fluoview FV100 laser-scanning confocal microscope to confirm colocalization with either NeuN or GFAP. In the $TR\alpha1^{+/m}$ mice, 50 $BrdU^+$ cells in each animal per marker were analyzed to confirm colocalization with either the transient neuronal marker calretinin or the mature neuronal marker NeuN, using *z*-plane sectioning on a Zeiss Axiovert 510LSM confocal laser-scanning microscope. Percentage colocalization of BrdU with either NeuN or calretinin was determined for $BrdU^+$ cells within the SGZ/GCL or directly in contact with the SGZ and was confirmed using *z*-plane stack analysis of confocal images.

In TRα1-GFP mice, 20 BrdU, NeuroD, or DCX-immunopositive cells per animal (n=3) were analyzed for colocalization with GFP using *z*-plane sectioning on a Zeiss Axiovert 510LSM confocal laser-scanning microscope.

Statistical analysis

Results were subjected to statistical analysis using the program Prism (GraphPad, San Diego, CA, USA). Experiments with two groups were analyzed for differences using the unpaired Student's t test, with significance determined at $P < 0.05$. Experiments with 4 groups were subjected to statistical analyses using analysis of variance (ANOVA) followed by the Bonferroni *post hoc* test, with significance determined at values of $P < 0.05$.

RESULTS

TRα1 is expressed in newborn neurons of the adult hippocampus

Given recent evidence that thyroid hormone regulates adult hippocampal neurogenesis (9–11) and that the most abundant thyroid hormone receptor isoform in the brain is TRα1 (16), we sought to further elucidate the function of this receptor in hippocampal neurogenesis. To examine the expression of the TRα1 receptor in adult hippocampal progenitors, we used a novel knock-in TRα1-GFP mouse strain generated by inserting the coding sequence of eGFP in frame with the

TRα1 gene. In the hippocampus, GFP expression was observed within nuclei of cells in the SGZ and the GCL of the DG subfield. Double immunohistochemistry for the mitotic marker BrdU and GFP was performed to determine whether TRα1 is expressed by the proliferating pool of adult hippocampal progenitors. The lack of colocalization of TRα1-GFP with BrdU in hippocampal progenitors (**Fig. 1*A***) indicated that this receptor was not expressed by proliferating progenitor cells within the SGZ in the DG. To address whether TRα1 is expressed later in neuronal development, *i.e.*, in the largely postmitotic pool of adult hippocampal progenitors, we performed double immunohistochemistry for GFP and 2 markers for immature neurons; NeuroD and DCX. Confocal analysis revealed that TRα1 was expressed in both NeuroD and DCX^+ progenitors in the SGZ and GCL within the DG (Fig. 1*B*, *C*). These results indicate that within the neurogenic niche of the adult hippocampus, TRα1 is predominantly expressed by postmitotic progenitors destined to acquire a neuronal fate, and does not appear to be present in proliferating progenitors. On the basis of these findings along with our previous results (10), we hypothesized that TRα1 plays a role in the survival of adult hippocampal progenitors, and used mice lacking or harboring a mutation in the TRα isoforms to further investigate this hypothesis.

Proliferation of adult hippocampal progenitors is unaffected in the DG of $TR\alpha1^{-/-}$ and $TR\alpha2^{-/-}$ mice

Male $TR\alpha1^{-/-}$ mice and wild-type littermate controls were injected with BrdU 2 h prior to sacrifice in order to assess the influence of TRα1 receptor loss on the proliferation of adult hippocampal progenitors (**Fig. 2**). Stereological analysis indicated no change in the number of $BrdU^+$ progenitors in the SGZ/GCL of $TR\alpha1^{-/-}$ mice as compared to wild-type animals (Fig. 2*A*, left graph). We also examined the expression of an endogenous marker of cell division, PCNA, and the number of $PCNA^+$ cells in the SGZ/GCL was unaltered in $TR\alpha1^{-/-}$ mice (Fig. 2*A*, right graph). We next examined the numbers of BrdU- and $PCNA^+$ cells in the SGZ/GCL of $TR\alpha2^{-/-}$ mice that overexpress TRα1 as a direct consequence of the gene-targeting strategy. $TR\alpha2^{-/-}$ mice do not exhibit any change in the number of BrdU- or $PCNA^+$ cells within the SGZ/GCL, indicating no effect in these mice on hippocampal progenitor proliferation (Fig. 2*B*, graphs). In both wild-type and TRα1- and TRα2-deficient mice, BrdU- and $PCNA^+$ cells were observed in clusters within the SGZ at the border of the GCL and the hilus.

$TR\alpha1^{-/-}$ and $TR\alpha2^{-/-}$ mice show opposing survival of adult hippocampal progenitors

To examine the postmitotic survival of adult hippocampal progenitors, $TR\alpha1^{-/-}$ and $TR\alpha2^{-/-}$ knockout mice and their respective wild-type controls received daily BrdU injections for 3 d and were sacrificed 28 d later. $TR\alpha1^{-/-}$ mice showed a significant increase in the number of $BrdU^+$ cells in the SGZ/GCL as compared to wild-type controls, which indicated an increased survival of adult hippocampal progenitors in the mutants (**Fig. 3*A*,** right panel). In striking contrast, $TR\alpha2^{-/-}$ mice demonstrated a significant decline in the number of persisting $BrdU^+$ cells in the SGZ/GCL (Fig. 3*B*, right panel). The surviving $BrdU^+$ cells exhibit a characteristic ovoid shape and are not observed in clusters but are seen dispersed through the GCL (Fig. 3, image panels).

$TR\alpha1^{-/-}$ and $TR\alpha2^{-/-}$ mice exhibit contrasting effects on the numbers of DCX^+ immature neurons in the adult DG

DCX is a microtubule-associated protein expressed by postmitotic and migratory adult hippocampal progenitors (12) and is a useful endogenous marker for adult hippocampal neurogenesis) . $TR\alpha1^{-/-}$ mice exhibited

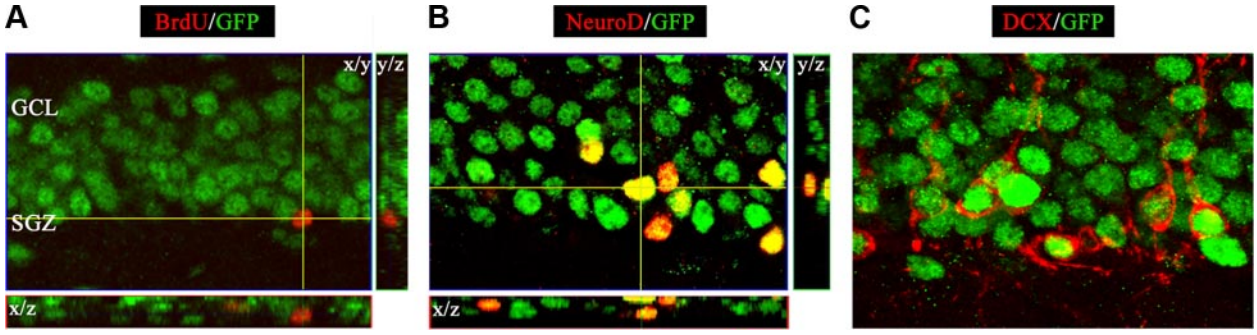

Figure 1. TRα1 is expressed in Neuro-D- and DCX^+ progenitors within the DG. TRα1-GFP-knock-in mice generated by insertion of the coding sequence of eGFP in frame with the TRα1 gene were utilized to study the expression of TRα1-GFP chimeric protein using double immunofluorescence studies. TRα1-GFP-knock-in mice received a single injection of BrdU (150 mg/kg, $n=3$) and were sacrificed 2 h later. *A*) Confocal *z*-stack image at ×630, indicating the lack of colocalization of TRα1-GFP with BrdU within the SGZ of the DG. *B*) $NeuroD^+$ progenitors within the SGZ and GCL of the DG colocalized with GFP, as shown in a representative confocal *z*-stack image (×630). *C*) DCX-immunopositive immature neurons were also observed to exhibit colocalization with GFP at ×630, indicating the presence of TRα1 in DCX^+ immature neurons.

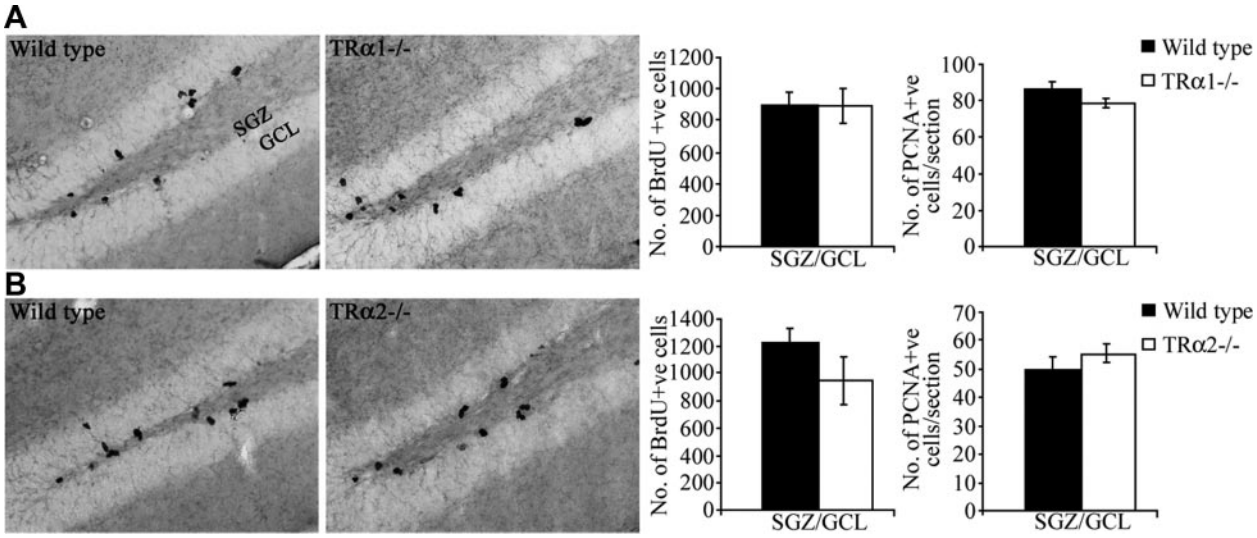

Figure 2. TRα1$^{-/-}$ and TRα2$^{-/-}$ mice do not exhibit any change in the proliferation of adult hippocampal progenitors. TRα1$^{-/-}$ and TRα2$^{-/-}$ mice and respective wild-type littermate controls received a single injection of BrdU (100 mg/kg, n=3–5/group) and were sacrificed 2 h later to assess the numbers of proliferating adult hippocampal progenitors in these mutant mice. Images show representative photomicrographs of BrdU$^+$ cells at ×200 from TRα1$^{-/-}$ (*A*) and TRα2$^{-/-}$ (*B*) mice (right images), along with respective wild-type controls (left images). BrdU$^+$ cells were predominantly observed in clusters within the SGZ, at the border of the hilus and the GCL. Left graphs: stereological analysis revealed no change in number of BrdU$^+$ cells within the SGZ/GCL in TRα1$^{-/-}$ (*A*) and TRα2$^{-/-}$ (*B*) mice, as compared to their respective wild-type controls. Right graphs: to examine ongoing adult hippocampal progenitor proliferation, we also used immunohistochemical analysis of an endogenous marker of cell proliferation, PCNA. Quantitative analysis did not indicate any change in numbers of PCNA$^+$ cells within the SGZ/GCL of TRα1$^{-/-}$ (*A*) and TRα2$^{-/-}$ (*B*) mice, as compared to respective wild-type controls. Results are expressed as the mean ± SE number of BrdU$^+$ cells in the SGZ/GCL (n=3–5/group), or number of PCNA$^+$ cells per section.

a significant increase in the number of DCX$^+$ cells/section within the SGZ/GCL (**Fig. 4*A*,** right panel), whereas TRα2$^{-/-}$ mice had reduced DCX$^+$ cell numbers as compared to their wild-type controls (Fig. 4 *B*, right panel). Taken together, the BrdU and DCX results indicate an increased hippocampal neurogenesis in TRα1$^{-/-}$ mice in contrast to the decrease seen in the TRα2$^{-/-}$ strain.

DCX immunohistochemistry marks both the cell body and the dendritic arbors of newborn neurons (Fig. 4*A*, *B*). As immature neurons undergo a morphological maturation, the dendritic tree becomes more elaborate with the presence of complex tertiary dendrites. As described previously (26), we subcategorized DCX$^+$ cells as those with or without tertiary dendrites (Fig. 4*C*, left panels). While numbers of DCX$^+$ cells were clearly different in TRα1$^{-/-}$ and TRα2$^{-/-}$ mice, we did not observe any change in the morphological maturation of these DCX$^+$ cells, as the percentage of DCX$^+$ cells with complex tertiary dendrites was unaltered in the mutants as compared to their controls (Fig. 4*C*, right panels).

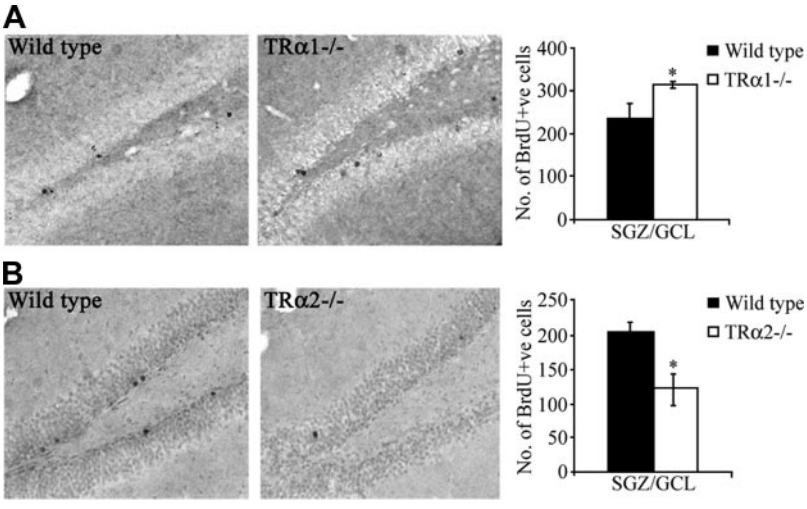

Figure 3. TRα1$^{-/-}$ mice exhibit an increase in survival of BrdU$^+$ adult hippocampal progenitors, whereas TRα2$^{-/-}$ mice show a decrease in survival of BrdU$^+$ progenitors. TRα1$^{-/-}$ and TRα2$^{-/-}$ mice and respective wild-type littermate controls received BrdU (150 mg/kg) once daily by intraperitoneal injection for 3 d and were sacrificed 28 d after final BrdU injection. Images show representative photomicrographs of BrdU$^+$ cells from TRα1$^{-/-}$ (*A*) and TRα2$^{-/-}$ (*B*) mice (right panels), along with respective wild-type littermate controls (left panels) at ×200. BrdU$^+$ cells within the SGZ and the GCL were ovoid in morphology and rarely observed in clusters. Right panels: quantitative stereological analysis showed that TRα1$^{-/-}$ knockout mice had significantly higher numbers of surviving BrdU$^+$ cells in the SGZ/GCL (*A*), whereas TRα2$^{-/-}$ knockout mice showed a significant decrease in numbers of BrdU$^+$ cells in the SGZ/GCL (*B*). Results are expressed as the mean ± SE number of BrdU$^+$ cells in the SGZ/GCL (n=3–5/group). *$P < 0.05$ *vs.* wild-type control (Student's t test).

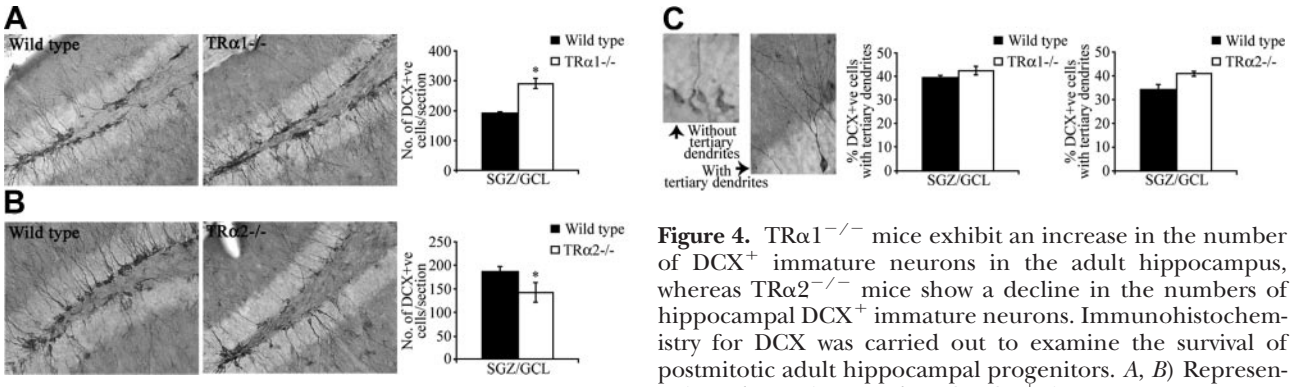

Figure 4. TRα1$^{-/-}$ mice exhibit an increase in the number of DCX^{+} immature neurons in the adult hippocampus, whereas TRα2$^{-/-}$ mice show a decline in the numbers of hippocampal DCX^{+} immature neurons. Immunohistochemistry for DCX was carried out to examine the survival of postmitotic adult hippocampal progenitors. *A*, *B*) Representative photomicrographs of DCX^{+} immature neurons at ×200 within the SGZ/ GCL in the DG subfield in TRα1$^{-/-}$ (*A*) and TRα2$^{-/-}$ (*B*) mice (right images), along with respective wild-type controls (left images). Quantitative analysis revealed that TRα1$^{-/-}$ mice exhibit a significant increase in the number of DCX^{+} cells in the SGZ/GCL, as compared to wild-type controls (*A*, right panel). In striking contrast, TRα2$^{-/-}$ mice showed a significant decline in the number of DCX^{+} cells in the SGZ/GCL, as compared to wild-type controls (*B*, right panel). *C*) Analysis of the dendritic morphology of DCX^{+} cells revealed no change in the morphological maturation of DCX^{+} cells in TRα1$^{-/-}$ and TRα2$^{-/-}$ mice as compared to their wild-type littermate controls. Left panels: representative images of DCX^{+} cells at ×400, categorized as with or without tertiary dendrites. Right panels: Quantitative analysis of percentage distribution of DCX^{+} cells with tertiary dendrites in the SGZ/GCL revealed no change in TRα1$^{-/-}$ (left graph) and TRα2$^{-/-}$ (right graph) mice, as compared to wild-type controls. Results are expressed as mean ± SE number of DCX^{+} cells per section in the SGZ/GCL, or mean ± SE percentage of DCX^{+} cells with tertiary dendrites in the SGZ/GCL (n=3–5/group). *$P < 0.05$ *vs.* wild-type control (Student's t test).

TRα1$^{-/-}$ and TRα2$^{-/-}$ mice show an altered expression of markers of the neurogenic niche

Immunohistochemical analyses were performed to determine whether TRα1$^{-/-}$ and TRα2$^{-/-}$ mice exhibit changes in expression of additional stage-specific markers for adult hippocampal neurogenesis, such as PSA-NCAM and stathmin, which have previously been shown to colocalize with DCX expression (13, 27, 28). Similar to what was seen with DCX, TRα1$^{-/-}$ mice exhibited a significant increase, whereas TRα2$^{-/-}$ mice showed a significant decline in the number of cells positive for PSA-NCAM (TRα1$^{-/-}$: **Fig. 5*A***, TRα2$^{-/-}$: Fig. 5*B*) and stathmin (TRα1$^{-/-}$: Fig. 5*C*, TRα2$^{-/-}$: Fig. 5*D*) as compared to their wild-type controls. While PSA-NCAM expression (Fig. 5*A*, *B*) was noted in both the cell body, as well as the dendritic arbor similar to DCX expression, stathmin expression (Fig. 5*C*, *D*) was predominantly observed in the cell body. Taken together, our results demonstrate that TRα1$^{-/-}$ mice show a significant increase, while TRα2$^{-/-}$ mice exhibit a decline, in the number of DCX-, PSA-NCAM- and stathmin-positive cells within the adult hippocampal neurogenic niche.

We also sought to address whether the TRα1$^{-/-}$ and TRα2$^{-/-}$ mice exhibit any gross changes in hippocampal and GCL volume using modified stereological approaches (10). We observed no significant change in either the total hippocampal volume or in the volume of the GCL in the DG hippocampal subfield in the TRα1$^{-/-}$ or TRα2$^{-/-}$ mice, as compared to their respective littermate wild-type controls (Supplemental

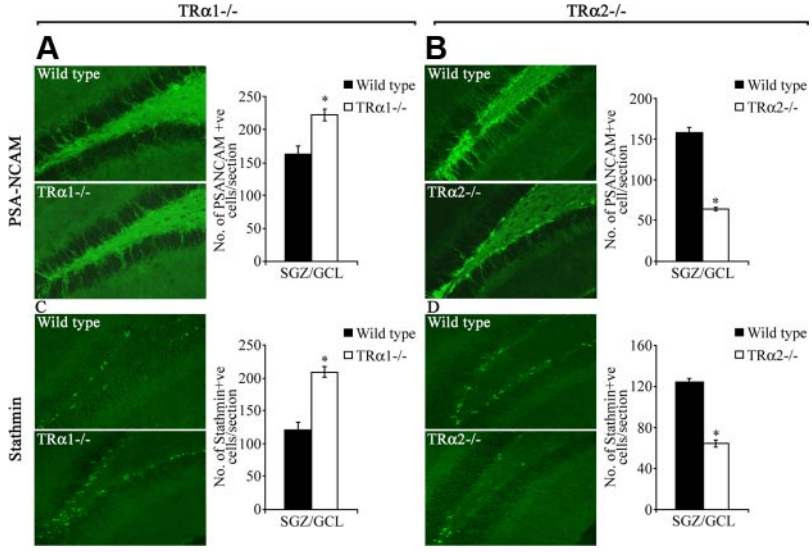

Figure 5. TRα1$^{-/-}$ and TRα2$^{-/-}$ mice display altered expression of neurogenic niche markers. Numbers of PSA-NCAM and stathmin-immunopositive cells in TRα1$^{-/-}$ and TRα2$^{-/-}$ mice were assessed using immunofluorescence. Panels show representative immunofluorescence images for PSA-NCAM (*A*, *B*) and stathmin (*C*, *D*) from TRα1$^{-/-}$ (*A*, *C*) and TRα2$^{-/-}$ mice (*B*, *D*) and respective wild-type controls at ×200. Quantitative analysis indicated a significant increase in numbers of both PSA-NCAM- and stathmin-positive cells in the SGZ/GCL of the TRα1$^{-/-}$ mice *vs.* wild-type controls (*A*, *C*). In contrast, a significant decline in numbers of both PSA-NCAM- and stathmin-positive cells per section was observed in TRα2$^{-/-}$ mice *vs.* wild-type controls (*B*, *D*). Results are expressed as mean ± SE number of PSA-NCAM- or stathmin-positive cells per section in the SGZ/GCL (n=3–5/group). *$P < 0.05$ *vs.* wild-type control (Student's t test).

Fig. 1). Further, granule cell neuron marker (Prox1) expression, mossy fiber bundle innervation of the CA3, and GFAP-immunopositive glial cell numbers in the DG subfield were found to be unaltered in the TRα1$^{-/-}$ or TRα2$^{-/-}$ mice as compared to their respective littermate wild-type controls (Supplemental Figs. 2 and 3).

Differentiation of newborn progenitors is not perturbed in TRα1$^{-/-}$ and TRα2$^{-/-}$ mice

To address whether cell fate acquisition by newborn adult hippocampal progenitors is altered in TRα1$^{-/-}$ and TRα2$^{-/-}$ mice, we examined the percentage of colocalization of BrdU with the neuronal marker NeuN or the glial marker GFAP, 28 d after BrdU administration using triple immunofluorescence and confocal microscopy. Quantitative analysis, based on confocal *z*-stack analysis to detect colocalization, revealed no change in the percentage of colocalization of BrdU with NeuN or GFAP in both TRα1$^{-/-}$ and TRα2$^{-/-}$ mice, as compared to the wild-type controls (**Fig. 6*B*, *C***). In wild type, as well as TRα1$^{-/-}$ and TRα2$^{-/-}$ mice, more BrdU^{+} cells acquired a neuronal, rather than a glial, fate. BrdU^{+} cells that do not colocalize with either NeuN or GFAP may represent as yet undifferentiated cells.

Thyroid hormone administration to TRα2$^{-/-}$ mice rescues the decline in the number of DCX^{+} immature neurons

We hypothesized that the decreased survival of BrdU^{+} progenitors and the decline in DCX, PSA-NCAM, and stathmin cell numbers in TRα2$^{-/-}$ mice may reflect a limited availability to ligand for the overexpressed TRα1, thus causing an aporeceptor effect in the neurogenic niche similar to those described previously for other tissues (18). To test this hypothesis, we treated the TRα2$^{-/-}$ mice with excess thyroid hormone. Animals were divided into 4 groups, with TRα2$^{-/-}$ mice and their wild-type controls receiving either vehicle or thyroid hormone in drinking water for 16 d prior to sacrifice. We then assessed whether the hormone treatment could rescue the decrease in number of hippocampal progenitors expressing the endogenous marker, DCX, in the TRα2$^{-/-}$ mice. Thyroid hormone treatment was capable of completely rescuing the decline in DCX^{+} cell number in the SGZ/GCL of TRα2$^{-/-}$ mice (**Fig. 7*B***). Interestingly, thyroid hormone treatment also significantly enhanced the number of DCX^{+} cells in the SGZ/GCL of wild-type animals. These results support the hypothesis that the decrease in progenitor survival observed in TRα2$^{-/-}$ mice, is a consequence of a TRα1 aporeceptor effect, which can be ameliorated through the restoration of an appropriate ligand-receptor ratio *via* thyroid hormone treatment.

Rescue of survival and neuronal differentiation of adult hippocampal progenitors in TRα1$^{+/m}$ mice by thyroid hormone administration

To further address the hypothesis that an unliganded TRα1 results in a decline in adult hippocampal neurogenesis, we used mice expressing a mutant TRα1 with dominant-negative activity. These heterozygous mice (TRα1$^{+/m}$) were generated by introducing a point mutation in the TRα1 gene (TRα1R384C) that lowers the affinity for thyroid hormone 10-fold (20). We first studied the effects of reduced ligand-binding in TRα1$^{+/m}$ mice on adult hippocampal progenitor proliferation. Wild-type controls and TRα1$^{+/m}$ mice received a single BrdU injection and were sacrificed 2 h later. BrdU immunohistochemistry followed by stereological quantitative analysis showed that mutant mice did not differ from wild-type animals in the number of BrdU^{+} cells; these results were corroborated by PCNA immunohistochemistry (**Fig. 8*A*, *B***).

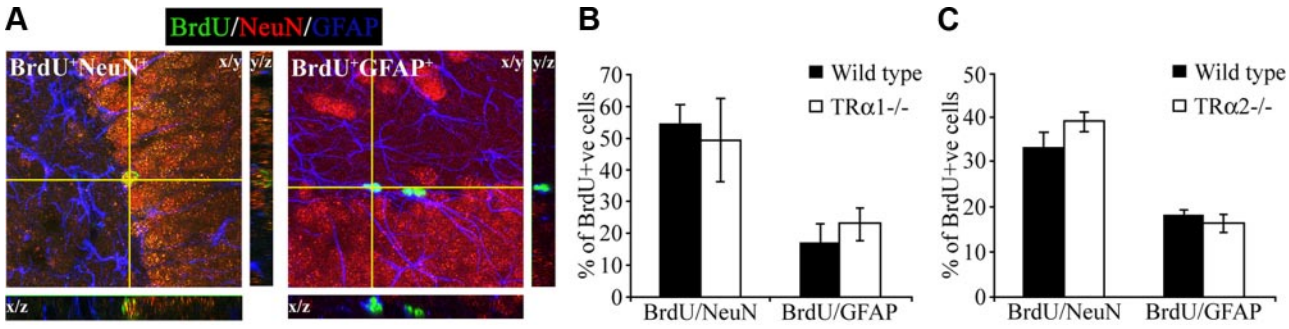

Figure 6. Differentiation of adult hippocampal progenitors into NeuN^{+} neurons or GFAP^{+} glia is unaffected in TRα1$^{-/-}$ and TRα2$^{-/-}$ mice. TRα1$^{-/-}$ and TRα2$^{-/-}$ mice, along with their respective wild-type littermate controls, received BrdU (150 mg/kg) once daily by intraperitoneal injection for 3 d and were sacrificed 28 d after the last injection. To examine the neuronal and glial differentiation of newborn adult hippocampal progenitors within the SGZ/ GCL triple immunofluorescence for BrdU, the mature neuronal marker, neuronal nuclei (NeuN), and the glial marker, GFAP was performed. *A*) Representative merged confocal *z*-stack images of BrdU colocalization with NeuN (BrdU^{+}NeuN^{+}) or GFAP (BrdU^{+}GFAP^{+}), with the cell at the crosshairs demonstrating colocalization (×600). *B, C*) Confocal *z*-stack analysis indicated no change in colocalization of BrdU with either NeuN or GFAP in the TRα1$^{-/-}$ (*B*) or the TRα2$^{-/-}$ (*C*) mice *vs.* wild-type controls. Results are expressed as mean ± SE percentage colocalization of BrdU^{+} cells with NeuN or GFAP in the SGZ/GCL (n=3–5/group).

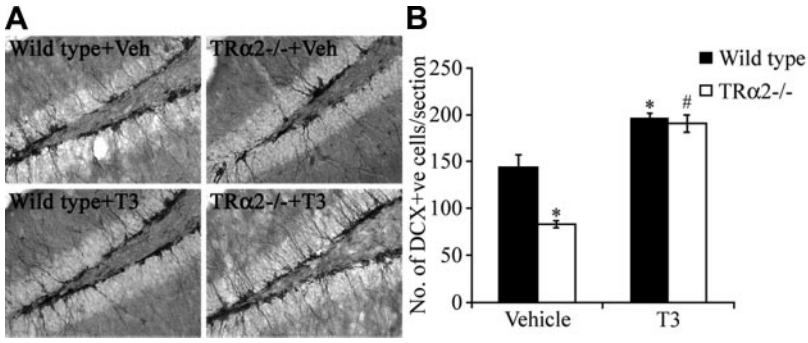

Figure 7. Thyroid hormone treatment rescues the decrease in number of DCX^+ immature neurons in $TR\alpha2^{-/-}$ mice. Wild-type and $TR\alpha2^{-/-}$ mice were divided into 4 experimental groups (wild type + veh, wild type + T3, $TR\alpha2^{-/-}$ + veh, and $TR\alpha2^{-/-}$ + T3). The groups receiving thyroid treatment were given T3 (0.5 μg/ml) in drinking water for 16 d prior to sacrifice. *A*) Representative photomicrographs (×200) of DCX immunohistochemistry in the SGZ/GCL of vehicle- and T3-treated wild-type and $TR\alpha2^{-/-}$ mice. *B*) Stereological quantitative analysis revealed a significant decline in the number of cells expressing DCX in the vehicle-treated $TR\alpha2^{-/-}$ mice *vs.* vehicle-treated wild-type controls. This decrease was rescued by thyroid hormone treatment, as the number of DCX^+ cells in the T3-treated $TR\alpha2^{-/-}$ mice was significantly higher than vehicle-treated $TR\alpha2^{-/-}$ mice. There was also a significant increase in the number of DCX^+ cells in the SGZ/GCL of T3-treated wild-type mice *vs.* vehicle treated wild-type controls. Results are expressed as mean ± SE number of DCX^+ cells per section in the SGZ/GCL (n=4 or 5/group). $^*P < 0.05$ *vs.* vehicle-treated wild-type control group; $^{\#}P < 0.05$ *vs.* vehicle-treated $TR\alpha2^{-/-}$ group (ANOVA and Bonferroni *post hoc* test).

To examine the survival of $BrdU^+$ progenitors in the hippocampus, wild-type controls and $TR\alpha1^{+/m}$ mice were injected with BrdU once daily for 3 d and sacrificed 30 d after the last BrdU injection. As compared to wild-type mice, a significant decrease in the number of surviving $BrdU^+$ cells was seen in $TR\alpha1^{+/m}$ mice. Thyroid hormone treatment of $TR\alpha1^{+/m}$ mice normalized the numbers of surviving $BrdU^+$ cells as compared to vehicle-treated wild-type controls (Fig. 8*D*). Interestingly, thyroid hormone treatment to wild-type controls itself resulted in an increased number of $BrdU^+$ cells that persisted 30 d after BrdU injection. Taken together, with the results obtained from the $TR\alpha2^{-/-}$ mice, the data indicate that an unliganded TRα1 receptor could function as an inhibitor of adult hippocampal neurogenesis through an effect on the survival of newborn hippocampal progenitors.

To determine whether the unliganded TRα1 influences the differentiation of $BrdU^+$ progenitors into mature neurons, double-labeling studies were performed using antibodies to BrdU and NeuN. Confocal analysis revealed a significant reduction in the percentage of $BrdU^+$ cells that are immunopositive for the neuronal marker NeuN (Fig. 8*E*) indicating that fewer progenitor cells acquire a neuronal phenotype in $TR\alpha1^{+/m}$ mice. Thyroid hormone treatment ameliorated the decrease in neuronal differentiation observed in $TR\alpha1^{+/m}$ mutant mice (Fig. 8*E*). However, thyroid hormone treatment to wild-type mice did not influence neuronal differentiation. These results suggest that unliganded TRα1 influences both hippocampal progenitor cell survival and neuronal differentiation.

Given the decline observed in BrdU/NeuN colocalization in $TR\alpha1^{+/m}$ mice, we next sought to address whether the expression of calretinin in $BrdU^+$ postmitotic hippocampal progenitors is also altered in $TR\alpha1^{+/m}$ mice. Calretinin, a calcium-binding protein, is transiently expressed in the postmitotic stages of hippocampal progenitor cell development (29), and calretinin-immunopositive cells represent a fraction of immature, postmitotic adult hippocampal progenitors that are destined for neuronal differentiation. Calretinin expression is thought to precede the expression of the mature neuronal marker NeuN. Confocal analysis revealed a significant decline in the colocalization of BrdU with calretinin in the $TR\alpha1^{+/m}$ mice, which was partially normalized by thyroid hormone treatment (Fig. 8*F*).

Decrease in $NeuroD^+$ cells in the hippocampal neurogenic niche of $TR\alpha2^{-/-}$ and $TR\alpha1^{+/m}$ mice

The transcription factor NeuroD is required for the differentiation of dentate granule cells of the hippocampus (30) and is expressed in adult hippocampal progenitors (31, 32). We examined by immunohistochemistry the number of $NeuroD^+$ cells within the SGZ/GCL of $TR\alpha2^{-/-}$ and $TR\alpha1^{+/m}$ mice. In wild-type mice, NeuroD was strongly expressed in the SGZ where adult hippocampal progenitors reside, whereas the expression was substantially lower in the GCL (**Fig. 9*A, C***). In both mutant mouse strains we observed a significant reduction in the number of NeuroD-immunopositive cells within the SGZ/GCL as compared to their respective wild-type controls (Fig. 9). Thyroid hormone treatment normalized the number of $NeuroD^+$ cells in $TR\alpha1^{+/m}$ mice (Fig. 9*D*). In the $TR\alpha2^{-/-}$ mice, thyroid hormone treatment showed a trend to increase the number of $NeuroD^+$ cells/section; however, this did not reach significance (P=0.06, ANOVA and Bonferroni *post hoc* test; Fig. 9*B*). Interestingly, thyroid hormone treatment resulted in a significant increase in $NeuroD^+$ cell number in wild-type control littermates from both the $TR\alpha2^{-/-}$ and $TR\alpha1^{+/m}$ experiments (Fig. 9*B, D*).

DISCUSSION

Our aim in this study was to address the role of TRα1 in adult hippocampal neurogenesis using $TR\alpha1^{-/-}$, $TR\alpha2^{-/-}$, and $TR\alpha1^{+/m}$ mutant mice. We provide novel evidence that TRα1 regulates adult hippocampal neurogenesis, through effects on progenitor survival and neuronal differentiation. The striking similarity

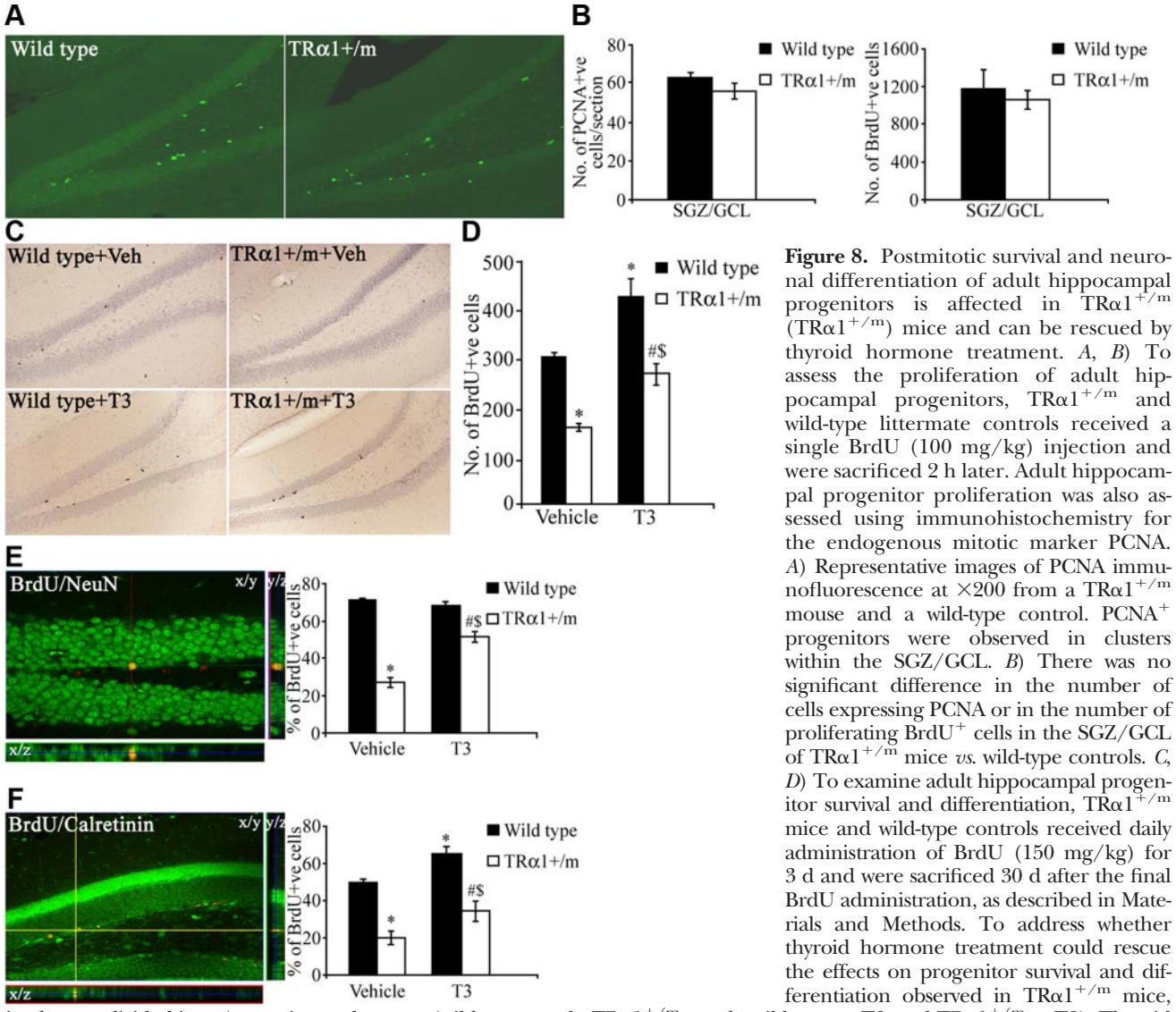

Figure 8. Postmitotic survival and neuronal differentiation of adult hippocampal progenitors is affected in TRα1$^{+/m}$ (TRα1$^{+/m}$) mice and can be rescued by thyroid hormone treatment. *A*, *B*) To assess the proliferation of adult hippocampal progenitors, TRα1$^{+/m}$ and wild-type littermate controls received a single BrdU (100 mg/kg) injection and were sacrificed 2 h later. Adult hippocampal progenitor proliferation was also assessed using immunohistochemistry for the endogenous mitotic marker PCNA. *A*) Representative images of PCNA immunofluorescence at ×200 from a TRα1$^{+/m}$ mouse and a wild-type control. PCNA^{+} progenitors were observed in clusters within the SGZ/GCL. *B*) There was no significant difference in the number of cells expressing PCNA or in the number of proliferating BrdU^{+} cells in the SGZ/GCL of TRα1$^{+/m}$ mice *vs.* wild-type controls. *C*, *D*) To examine adult hippocampal progenitor survival and differentiation, TRα1$^{+/m}$ mice and wild-type controls received daily administration of BrdU (150 mg/kg) for 3 d and were sacrificed 30 d after the final BrdU administration, as described in Materials and Methods. To address whether thyroid hormone treatment could rescue the effects on progenitor survival and differentiation observed in TRα1$^{+/m}$ mice, animals were divided into 4 experimental groups (wild type + veh, TRα1$^{+/m}$ + veh, wild type + T3, and TRα1$^{+/m}$ + T3). Thyroid hormone treatment was administered in drinking water as described in Materials and Methods. *C*) Representative photomicrographs of BrdU immunohistochemistry at ×200 in the SGZ/GCL of vehicle and T3-treated wild-type and TRα1$^{+/m}$ mice. *D*) Number of BrdU^{+} cells that persist 30 d after BrdU administration in the SGZ/GCL of TRα1$^{+/m}$ mice was significantly lower than that observed in vehicle-treated wild-type controls. T3-treatment restored the decrease in postmitotic survival in the TRα1$^{+/m}$ mice and also significantly increased the number of BrdU^{+} cells in the SGZ/GCL of wild-type mice compared to vehicle-treated wild-type controls. *E*, *F*) Neuronal differentiation was assessed by examining the colocalization of BrdU with two markers of neuronal differentiation, NeuN and calretinin. Microphotos show representative merged confocal *z*-stack images of BrdU-NeuN colocalization (*E*) and BrdU-calretinin colocalization (*F*) at ×400. Crosshairs indicate examples of BrdU^{+} cells that colocalize with NeuN (*E*) and calretinin (*F*), with orthogonal sections confirming colocalization in the *z* plane. Percentage colocalization of BrdU with NeuN (*E*) and with calretinin (*F*) was significantly reduced in TRα1$^{+/m}$ mice *vs.* wild-type controls. Thyroid hormone treatment was capable of partially rescuing the decline in BrdU-NeuN and BrdU-calretinin colocalization observed in TRα1$^{+/m}$ mice. Results are expressed as mean ± SE number of BrdU^{+} cells (*B*, *D*), number of PCNA^{+} cells per section (*B*), or percentage colocalization of BrdU^{+} cells with NeuN (*E*) or calretinin (*F*) in the SGZ/GCL (n=4 or 5/group). *$P < 0.05$ *vs.* vehicle-treated wild-type controls; $^{\#}P < 0.05$ *vs.* T3-treated wild-type controls; $^{\$}P < 0.05$ *vs.* vehicle treated TRα1$^{+/m}$ group (ANOVA and Bonferroni *post hoc* test).

between the decreased adult neurogenesis observed in hypothyroidism (9, 10), and our results of the neurogenic phenotype in mutant mice with TRα1 aporeceptor activity, strongly suggest that an unliganded TRα1 contributes to the neurogenic decline in adult-onset hypothyroidism.

While the requirement for thyroid hormone during neurodevelopment is well established (33, 34), it is only recently that the effects of thyroid hormone on adult neurogenesis have been reported (9, 10, 11, 35). Impaired adult hippocampal neurogenesis is implicated as a key structural correlate that contributes to the functional deficits in hippocampus-dependent learning and mood-related behaviors observed in rodents and humans with adult-onset hypothyroidism (4, 11, 36). However, the role of individual TRs in adult neurogenesis

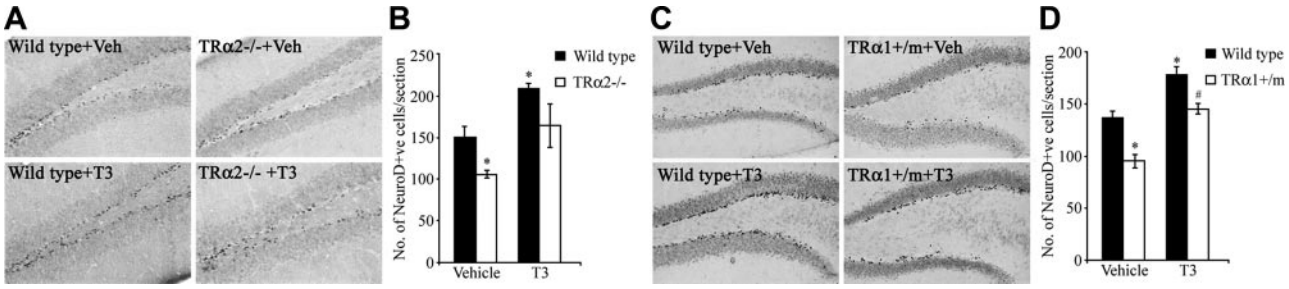

Figure 9. TRα2$^{-/-}$ and TRα1$^{+/m}$ mice exhibit a significant reduction in number of NeuroD^{+} cells within the SGZ/GCL. To assess the effect of thyroid hormone treatment on the number of NeuroD^{+} cells in TRα2$^{-/-}$ and TRα1$^{+/m}$ mice, treatment groups received T3 (0.5 μg/ml) in drinking water, as described in Materials and Methods. *A, C*) Representative photomicrographs of NeuroD-expressing cells at ×200 in the SGZ/ GCL of vehicle- and T3-treated TRα2$^{-/-}$ mice (*A*) and vehicle- and T3-treated TRα1$^{+/m}$ mice (*C*) and respective wild-type controls. *B, D*) There was a significant decrease in the number of NeuroD^{+} adult hippocampal progenitors in the SGZ/GCL of the TRα2$^{-/-}$ knockout mice *vs.* wild-type controls, which was partially rescued by thyroid hormone treatment but did not reach significance (P=0.06, Bonferroni *post hoc* test) (*B*). Similarly, there was a significant decline in number of NeuroD-expressing cells in the SGZ/GCL of vehicle-treated TRα1$^{+/m}$ mice *vs.* vehicle-treated wild-type controls, which was rescued by thyroid treatment (*D*). T3-treated wild-type mice also showed a significant increase in the number of NeuroD^{+} cells compared to vehicle-treated wild-type controls (*B, D*). Results are expressed as the mean ± SE number of NeuroD^{+} cells per section (n=3–5/group). *$P < 0.05$ *vs.* vehicle-treated wild-type control; $^{\#}P < 0.05$ *vs.* T3-treated wild-type control (ANOVA and Bonferroni *post hoc* test).

remains largely unidentified, with a single report (35) suggesting a role for TRα isoforms in the regulation of progenitor cell cycling in the subventricular zone lining the lateral ventricles.

Approximately 75% of thyroid hormone binding by TRs in the adult rodent brain is accounted for by TRα1 (16, 37). Immunohistochemical evidence indicates TRα isoform expression in adult hippocampal progenitors (10), but their stage-specific expression is unknown. Our colocalization studies of the TRα1-GFP chimeric protein with stage-specific progenitor markers indicate TRα1 expression in NeuroD and DCX-immunopositive progenitors, but not in proliferating BrdU^{+} progenitors. Our unpublished results with the TRα1-GFP-knock-in mice indicate that TRα1 expression is restricted to postmitotic stages of neuronal progenitor development. Further, proliferation was not altered in TRα1$^{-/-}$ mice, TRα2$^{-/-}$ mice, or TRα1$^{+/m}$ mutant mice. Taken together, these results imply that neither TRα1 nor TRα2 influence adult hippocampal progenitor proliferation. This is interesting in light of previous evidence (9, 10) that indicate no change in progenitor turnover following hypothyroidism or hyperthyroidism, suggesting a role for thyroid hormone in the postmitotic stages of adult hippocampal progenitor development.

TRα1$^{-/-}$ mice exhibited an increased survival of adult hippocampal progenitors, in striking contrast to the TRα1-overexpressing TRα2$^{-/-}$ mice that show a significant decline in progenitor survival. It could be argued that the decrease in postmitotic progenitor survival in the TRα2$^{-/-}$ mice is a consequence of TRα1 overexpression, or TRα2 loss, or a combination of both. However, we hypothesized, on the basis of the previously described phenotype of the TRα2$^{-/-}$ mice (18) that the overexpression of TRα1 may result in aporeceptor activity due to limited availability to ligand, and therefore reduced postmitotic survival of adult hippocampal progenitors. Strong support for this hypothesis comes from our results with the TRα1$^{+/m}$ mice that express a mutant TRα1 with aporeceptor activity, which show a robust decline in adult hippocampal progenitor survival. Both the TRα2$^{-/-}$ and TRα1$^{+/m}$ mice show a phenotype similar to hypothyroidism with decreased hippocampal progenitor survival, adding credence to the hypothesis that a TRα1 aporeceptor contributes to the deleterious consequences of hypothyroidism on adult hippocampal neurogenesis (**Fig. 10**). Interestingly, TRα1 deficiency enhances hippocampal progenitor survival suggesting the possibility that in the TRα1$^{-/-}$mice, besides the loss of liganded TRα1, the removal of a strong repressor activity of unliganded TRα1 may underlie the increased progenitor survival.

The cellular mechanisms for the effects of TRα1 on hippocampal progenitor survival are at present unclear. However, it is tempting to draw parallels with the proposed mechanisms for the actions of TRα1 on embryonic neuronal progenitors. Unliganded TRα1 has been suggested to arrest neuronal progenitors at specific stages of development until ligand availability allows them to progress further into neuronal differentiation (38). Adult hippocampal progenitors may recapitulate the effects of TRα1 aporeceptors observed in their developmental counterparts, with the TRα1 aporeceptor affecting expression of genes relevant for the progression of adult hippocampal progenitors to the next developmental stage. Indeed in both the TRα2$^{-/-}$ and TRα1$^{+/m}$ mice, the decline in progenitor survival suggests that TRα1 aporeceptors may result in postmitotic progenitors being arrested during maturation, with an eventual cell loss in the absence of a rescue by the ligand. Further support for this idea comes from our observations that thyroid hormone administration to TRα2$^{-/-}$ and TRα1$^{+/m}$ mice rescues the decreased survival observed using either an endogenous marker (DCX) or exogenous label (BrdU). The data also

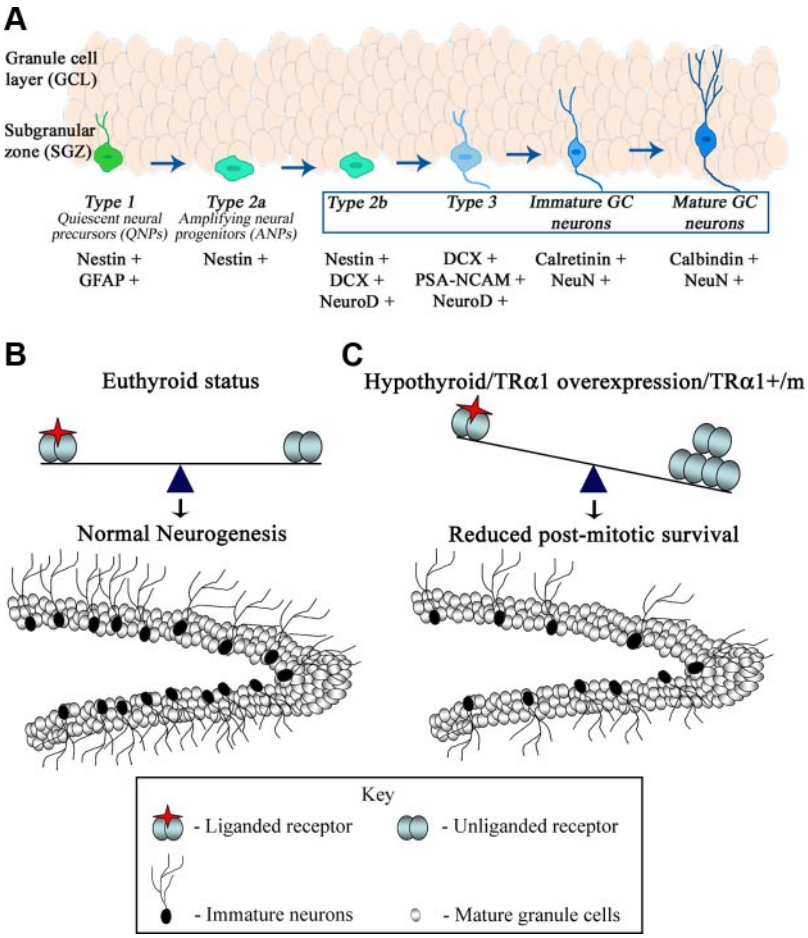

Figure 10. *A*) Schematic of the developmental stage-specific expression of markers by adult hippocampal progenitors. Type 1 quiescent neural precursors (QNPs) express nestin and GFAP and are the putative stem cells in the adult hippocampus. Type 2a cells, the transiently amplifying neural progenitors (ANPs), express Nestin alone, while Type 2b cells express nestin along with DCX and NeuroD. Type 3 neuroblasts express DCX, PSA-NCAM, and NeuroD, while immature neurons express calretinin and NeuN. Mature granule cell neurons express calbindin and NeuN. Boxed area denotes the stages of hippocampal progenitor development that are sensitive to perturbations of TRα1. *B, C*) Schematics of adult hippocampal neurogenesis in an euthyroid animal and in conditions that result in unliganded TRα1 receptor. Schematics show the DG subfield of the hippocampus, containing mature granule cell neurons (white) within the GCL, the hilus, and the neurogenic niche, namely the SGZ at the border of the hilus and the GCL, which contains the adult hippocampal progenitors. Postmitotic neuroblasts (black) in the SGZ arise from cell division of adult hippocampal progenitors and eventually differentiate into mature granule cells (white) within the GCL of the DG. *B*) Schematic of normal, ongoing hippocampal neurogenesis, which occurs in euthyroid conditions. *C*) Mice with conditions such as hypothyroidism, TRα2$^{-/-}$ mice that overexpress TRα1, and dominant-negative TRα1 (TRα1$^{+/m}$) mutant mice with a 10-fold reduction in affinity for thyroid hormone all show a decline in adult hippocampal neurogenesis, through a decrease in the survival of postmitotic neuroblasts. These effects are thought to arise as a consequence of repressor effects on the postmitotic survival of adult hippocampal progenitors induced by a TRα1 aporeceptor. A change in the ratio of unliganded and liganded TRα1 receptors may contribute *via* TRα1 aporeceptor effects to the impaired adult hippocampal neurogenesis observed in adult-onset hypothyroidism.

indicate that thyroid hormone treatment to wild-type mice increases hippocampal progenitor survival. Our results lead us to speculate that the effects of thyroid hormone on adult hippocampal neurogenesis may largely involve an alleviation of the TRα1 aporeceptor effect. A question that remains at present is whether the actions of TRα1, either liganded or unliganded, on adult hippocampal progenitor survival involve cell autonomous or noncell autonomous effects *via* modulation of the neurogenic niche. We have previously shown that thyroid hormone does exert direct effects on hippocampal progenitors *in vitro* (10); however, the *in vitro* effects differ substantially from the effects of perturbed thyroid hormone levels *in vivo*, indicating that dispersed progenitor cultures do not recapitulate the *in vivo* neurogenic niche vis a vis responses to thyroid hormone. Future studies that address the influence of TRα1 on neurogenic niche trophic factor expression, deiodinase expression in the hippocampal neurogenic niche and on astrocytic release of regulatory factors would further our mechanistic understanding of the effects of TRα1 on hippocampal neurogenesis.

Besides its effects on hippocampal progenitor survival, adult-onset hypothyroidism also significantly decreases neuronal differentiation, an effect relieved by thyroid hormone treatment (9, 10). The TRα1$^{+/m}$ mice exhibit a significant decline in the survival and neuronal differentiation of hippocampal progenitors, both of which can be rescued by thyroid hormone. This strong phenotypic similarity suggests that unliganded TRα1 may account for all of the changes observed in hippocampal neurogenesis in hypothyroidism. However, it is important to note that TRα2$^{-/-}$ mice, that overexpress TRα1, despite a robust decline in progenitor survival show normal neuronal differentiation of hippocampal progenitors, indicating a difference from the TRα1$^{+/m}$ mice. This discrepancy is likely to be due to the weak aporeceptor activity of TRα1 in the TRα2$^{-/-}$ mice, as caused by the receptor overexpression, as compared to the relatively much stronger aporeceptor activity caused by the point mutation in the TRα1R384C mutant receptor. Other contributing factors may include the differences in TR stoichiometry in these mutant mice. Future studies addressing the regulation of key target genes that modulate hippocampal progenitor survival and neuronal differentiation may help to resolve these issues. The TRα1$^{-/-}$ mice that show enhanced hippocampal progenitor survival exhibit normal neuronal differentiation, suggesting that deficiency of this receptor may not be capable of

further enhancing the neuronal differentiation of adult hippocampal progenitors.

We further addressed the early stages of postmitotic hippocampal progenitor development using two different markers, calretinin and NeuroD. Calretinin is a transient marker of postmitotic hippocampal progenitors destined for neuronal differentiation (29). The TRα1$^{+/m}$ mice show decreased BrdU-calretinin percentage colocalization, which can be rescued by thyroid hormone. Thyroid hormone administration to wild-type mice enhances the percentage of BrdU-labeled progenitors that are calretinin-immunopositive. Since we observed an influence of TRα1 aporeceptors on the calretinin-positive stage of neuronal differentiation in adult hippocampal progenitors, we examined whether an earlier stage of fate choice, namely, the acquisition of neuronal fate through the transcription factor NeuroD is also influenced. We observed a robust decline in NeuroD^{+} cell numbers within the hippocampal neurogenic niche of both TRα1$^{+/m}$ and TRα2$^{-/-}$ mice, which could be rescued by thyroid hormone administration. NeuroD is expressed in adult hippocampal progenitor cells (31) and is essential for progenitor survival and neuronal differentiation (30, 39). Furthermore, NeuroD expression is regulated by thyroid hormone, and hypothyroidism reduces NeuroD expression in the developing rat cerebellum (40) and the adult hippocampus (unpublished results). There are strong parallels to the effects of thyroid hormone on myogenic differentiation, where the TRα1 aporeceptor represses the transcription of the myogenic transcription factor, MyoD (41). Our results suggest that TRα1 aporeceptor activity may hold hippocampal progenitors at the early postmitotic stage prior to the acquisition of NeuroD^{+} identity and the commitment to a neuronal fate. The ligand thyroid hormone could act to allow progression of neuronal differentiation, and on the basis of reports that thyroid hormone cooperates with neurotrophins during hippocampal development (42) may also modulate the sensitivity of these postmitotic progenitors to neurotrophins within the niche.

Previous reports demonstrate that TRα1$^{+/m}$ mice also exhibit depressive behavior (43), anxiety, and memory impairments that are rescued by thyroid hormone treatment (22). Given the role of adult hippocampal neurogenesis in hippocampal-dependent memory (7, 44), depression (8), and anxiety behavior (45), this highlights the possibility that decreased hippocampal neurogenesis as a consequence of TRα1 aporeceptor activity may contribute to the behavioral deficits observed in both hypothyroidism and TRα1$^{+/m}$ mutant mice. In contrast, the TRα1$^{-/-}$ mice exhibit enhanced fear-associated learning, a behavior known to require hippocampal neurogenesis (46). The increased neurogenesis that we observed in the TRα1$^{-/-}$ mice may contribute to the improved fear of learning. However, the TRα1$^{-/-}$ mice also exhibit enhanced anxiety behavior, which has a more controversial association with neurogenesis, with increased anxiety behavior observed both following a decline or induction in hippocampal neurogenesis (47, 48). Our results motivate studies to determine the contribution of the neurogenic changes observed in the TRα1 mutants to the behavioral phenotypes observed in these mice.

In summary, we provide definitive evidence that TRα1 regulates adult hippocampal neurogenesis. Further, our data strongly indicate that TRα1 aporeceptor activity mediates the impaired survival and neuronal differentiation of adult hippocampal progenitors in adult-onset hypothyroidism. Our results highlight the requirement for future studies to identify the target genes that are regulated by both TRα1 apo- and holo-receptor activity, and their contribution to the decreased hippocampal neurogenesis and cognitive/behavioral deficits observed in adult-onset hypothyroidism. FJ

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