NIH Public Access
Author Manuscript
Behav Brain Res. Author manuscript; available in PMC 2008 February 12.

Published in final edited form as:
Behav Brain Res. 2007 February 12; 177(1): 109–116.

Behavioral Inhibition and Impaired Spatial Learning and Memory in Hypothyroid Mice Lacking Thyroid Hormone Receptor α

Jennifer Slone Wilcoxon[1,4], **Gregory J. Nadolski**[1,5], **Jacques Samarut**[2], **Olivier Chassande**[3], and **Eva E. Redei**[1]

1 *Northwestern University Feinberg School of Medicine, The Asher Center Department of Psychiatry and Behavioral Sciences, Chicago, IL 60611*

2 *Laboratoire de Biologie Moléculaire et Cellulaire de l'Ecole Normale Supérieure de Lyon, 69364 Lyon, France*

3 *Biomatériaux et Réparation Tissulaire INSERM U 443, Université de Bordeaux 2, Zone Nord, Batiment 4A, 2ème étage 146 rue Léo Saignat, 33076 Bordeaux, France*

Abstract

Thyroid hormone insufficiency leads to impaired neurogenesis, behavioral alterations and cognitive deficits. Thyroid hormone receptors, expressed in brain regions involved in these behaviors, mediate the effects of thyroid hormone deficiency or excess. To determine the contribution of thyroid hormone receptor alpha (TRα) in these behaviors, we examined the behavior of euthyroid as well as hypo- and hyperthyroid mice lacking all isoforms of the TRα ($TR\alpha^{o/o}$). The hypothyroxinemic $TR\alpha^{o/o}$ mice demonstrated behavioral inhibition, manifested in decreased activity and increased anxiety/fear in the open field test (OFT) and increased immobility in the forced swim test (FST) compared to C57BL/6J mice. $TR\alpha^{o/o}$ mice also showed learning and recall impairments in the Morris water maze (MWM), which were exaggerated by hypothyroidism in $TR\alpha^{o/o}$ mice. These impairments were concurrent with increased thigmotaxis, suggesting an increased anxiety-like state of the $TR\alpha^{o/o}$ mice in the MWM. Expression of genes, known to be involved in processes modulating learning and memory, such as glucocorticoid receptor (GR), growth-associated protein 43 (GAP-43) and neurogranin (RC3), were significantly decreased in the hippocampus of $TR\alpha^{o/o}$ mice. GR expression was also decreased in the frontal cortex and amygdala of $TR\alpha^{o/o}$ mice, indicating that expression of GR is regulated, probably developmentally, by one or more isoforms of TRα in the mouse brain. Taken together these data demonstrate behavioral alterations in the $TR\alpha^{o/o}$ mice, indicating the functional role of TRα, and a delicate interaction between TRα and TRβ-regulated genes in these behaviors. Thyroid hormone-regulated genes potentially responsible for the learning deficit found in $TR\alpha^{o/o}$ mice include GR, RC3 and GAP-43.

Keywords

Morris water maze; open field test; forced swim test; PTU; T4; glucocorticoid receptor; neurogranin; GAP-43

Corresponding Author: Eva E Redei, 303 E Chicago Ave, Ward 9-198, Chicago, IL 60611, Phone (312) 908-1791, Fax (312) 503-0466, Email: e-redei@northwestern.edu.
[4]*Present address*: Department of Neurobiology, Pharmacology and Physiology, The University of Chicago, IL, 60637, USA
[5]*Present address*: Indiana University School of Medicine, Indianapolis, Indiana 46202, USA

This research was supported by National Institute on Alcohol Abuse and Alcoholism Grant # AA07389 (ER) and #AA05587 (JSW)

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Introduction

In all vertebrates, thyroid hormones are essential for normal brain development [11,12,27, 61]. Experimentally induced moderate and transient deficiency of maternal thyroid hormones (TH) have profound effects on fetal neurogenesis [5] and brain growth; even mild hypothyroidism of the mother decreases maternal thyroxine (T4) transport to the fetus [57]. This mild maternal hypothyroidism can negatively affect the child's neuropsychological development [20,42], since the fetal thyroid is unable to produce any T4 before 12–14 weeks of gestation [42].

Research suggests that thyroid function abnormalities in adulthood can also have profound behavioral consequences. Clinical hypothyroidism is often associated with frank neuropsychological and affective alterations, and considered to be one of the causes of reversible dementia. Even mild hypothyroidism predicts lower levels of memory performance [55]. Additionally, moderate to severe depression and mania are associated with reduced TSH response to TRH [44].

In animals, hyperthyroidism in adulthood leads to increased activity in the open field test [46,50], and both experimental hypo- [25] and hyperthyroidism [46] have been reported to increase immobility behavior in the forced swim test. Spatial learning and memory deficits have been found in the genetically hypothyroid hyt/hyt mouse [3], and in congenitally hypothyroid animals [1,2,32].

Behavioral consequences of developmental or adult thyroid dysfunction are mediated by actions of thyroid hormones on thyroid hormone receptors (TR), predominantly by positive or negative transcriptional regulation of thyroid hormone responsive genes [39]. The ligand-activated nuclear TR isoforms, TRα1, TRβ1 and TRβ2, are produced by alternative splicing of the primary transcripts of the TRα and TRβ genes, respectively [52,58]. Another spliced product of the TRα gene, TRα2, does not bind TH [29]. The functions of the TR α1 and TR α2 isoforms, which are also expressed in the brain, are still unknown [31]. Recently, mice, lacking both TRα1 and TRα2 and the shorter transcripts initiated from the internal promoter located in intron 7, [15] have been generated offering the opportunity to characterize the role of these receptors in various behaviors. These TRα$^{o/o}$ mice have decreased T4, but normal TSH levels [16,31]. As such, the TRα$^{o/o}$ mice can model the mild hypothyroxemia, which leads to behavioral consequences, when it occurs during development and also in adulthood.

In this study, we aimed to determine the behavioral consequences of deletion of the TRα locus. To achieve this aim we employed TRα$^{o/o}$ mice and wild type C57BL/6J (B6) mice (the genetic background of the TRα$^{o/o}$ mice). Since we preferred to assure the normal thyroid milieu of the fetal C57BL/6J mice, we did not choose wild type littermates of the TRα$^{o/o}$ mice as controls. Instead, we considered the TRα$^{o/o}$ and C57BL/6J mice as two separate strains that differ genetically at their TRα locus, and developmentally in their thyroid hormonal milieu. To evaluate the role of the adult hypothyroxinemia on behaviors known to be affected by thyroid hormones, we induced hypo- or hyperthyroidism in both strains of adult mice. The behaviors examined included spatial learning and memory in the Morris Water Maze (MWM), activity and anxiety/fear in the open field test (OFT) and immobility in the forced swim test (FST). In addition, we evaluated the expression of GAP-43 and glucocorticoid receptor (GR), both implicated in learning [38,48,60], and the thyroid hormone-regulated gene, neurogranin (RC3), in brain regions involved in these behaviors.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Materials and Methods

Animals

Male C57BL/6J mice (Center for Comparative Medicine, Northwestern University, Illinois, USA) and mice with a homozygous null mutation for all forms of thyroid receptor alpha ($TR\alpha^{o/o}$) on a C57BL/6J background [31] were housed in a climate-controlled facility with a 12/12 light-dark cycle (lights on 7:00 h). $TR\alpha^{o/+}$ mice were crossed 11–13 times with C57BL/6J B6 mice before heterozygotes were intercrossed to result the $TR\alpha^{o/o}$ homozygotes (Dr. Samuel Refetoff, personal communication). After weaning, male mice were housed by strain (n=4–5/cage), and cages were composed of different litters. At six weeks of age, mice of both strains were placed on one of three diets. As a control diet, mice (n=13 mice/strain) were fed the standard rodent diet from Harlan Teklad (Madison, WI, USA). To elevate thyroid hormone levels, animals (n=13/strain) had unlimited access to water containing 0.0012% thyroxine (T4, Sigma T-2376) as described previously for rats [47]. To deplete thyroid hormone, mice (n=9/strain) were fed an iodine deficient diet containing 0.15% propylthiouracil (PTU, Harlan Teklad, Madison, WI, USA). The experimental diets were maintained throughout the study.

Behavioral tests were administered in the following order to the same animals, always in the morning between 1000h and 1200h. The OFT was administered first at 8 weeks of age, two weeks later the Morris water maze was performed, followed by the forced swim test after two weeks of rest.

Radioimmunoassay

Plasma T4 concentration was evaluated prior to behavioral testing and at the conclusion of the study. Blood was collected by tail nick four days prior to the beginning of the behavioral battery and again by heart puncture at the conclusion of the experiment. Total plasma T4 concentration was measured in a single T4 RIA kit from ICN Pharmaceuticals (Costa Mesa, CA, USA). The assay sensitivity was 0.3 μg/dl, and the intraassay and interassay coefficients of variation were 4.9% and 5.4%, respectively.

Open Field Test

Following two weeks of treatment with T4 or PTU-containing diet, mice were exposed to the open field test (OFT). The open field is a brightly lit circular arena with a total diameter of 60 cm and height of 30 cm. The arena is divided into inner and outer circles, where the diameter of the inner circle is 30 cm. Mice were placed in the center of the field, and their activity was recorded for 5 minutes. The test was recorded with a digital camera using the computer software Limelight from Actimetrix (Evanston, IL, USA). Limelight records the animal's latency to leave the center (a region in the center of the inner area with a 10 cm diameter), the time spent in the inner circle, the distance traveled in the inner circle, and the total distance traveled during the test.

Morris Water Maze

At 10 weeks of age, the mice were exposed to the MWM. The pool (d=108 cm) was filled with water (24–25^{o}C) and made opaque by non-toxic white tempera paint (Palmer Paint Products, Inc, Troy, MI, USA). The test consists of two days of 12 one-minute training trials, during which the mice attempted to locate a hidden platform submerged 1–2 cm below the surface of the water using visual cues located on the walls. The platform (d=10 cm) was placed in the middle of one of the quadrants of the pool. The training trials lasted one minute with a 5–6 minute rest period between trials. The trials began at one of four starting locations in bins of three. Thus, the first three training trials began from the same starting location; the next three trials began from a different starting location, and so on for the third and fourth sets of trials.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

The order of the starting locations was chosen at random and differed between the two days. When the mouse found the hidden platform, it remained on it for thirty seconds and then was removed from the pool. If the mouse was unable to locate the platform within sixty seconds, the animal was placed on the platform and remained there for thirty seconds. Following the final training trial on day two and a five-minute rest period, the mice underwent a one-minute probe trial in which the platform was removed from the pool, and the mice began from a unique starting location directly opposite the platform. During the probe trial, mice remained in the pool for the entire sixty seconds. Training ended at this point due to the plateau in the latency learning curve. All trials were recorded with a digital camera using the computer software WaterMaze from Actimetrix (Evanston, IL, USA). WaterMaze recorded the amount of thigmotaxis (% time spent < 5cm from the edge) and latency to platform of all trials. Additionally, WaterMaze recorded total distance traveled and the number of platform crossings during the probe trial.

Forced Swim Test

Between 13 and 15 weeks of age, mice were exposed to the forced swim test (FST). Mice were placed into water (23–25^{o}C) for 6 minutes. The FST tank was a beaker (d=13 cm) filled with water to a height of 12.5–13 cm. The test was videotaped and blindly scored using a method for rats described previously [53]. The animal was observed every five seconds beginning with the animal's entrance into the water and scored as either floating or swimming throughout the test. Floating was defined as the lack of movement of any limbs.

Brain Dissections

One week after the FST, mouse brains were rapidly dissected on ice and immediately placed on dry ice. Dissections used Paxinos coordinates [41]: amygdala (AP –0.58 to –2.18, ML 1.5 to 4.5, DV 4 to 5.75), frontal cortex (AP 3.2 to 2.34, ML 1.25 to 1.25, DV 0.6 to 2.25) and hippocampus (AP –0.58 to –2.18, ML 3.1 to 3.1, DV 1.1 to 2.75 and AP –2.3 to –3.16, ML 1.25 to 3.75, DV 1 to 5). Tissues were stored at −80°C.

RNA Isolation and Northern Analysis

Extraction of total RNA was performed using Trizol reagent (Life Technologies, Grand Island, NY, USA). The quality and quantity of RNA were analyzed by gel electrophoresis and spectrophotometry. For northern analysis, 8–10 μg of RNA from each sample was electrophoresed on a 1% agarose-formaldehyde gel, blotted onto a nitrocellulose filter, and fixed by UV-crosslinking. Probes were labeled with [α-32P]dCTP by random primer labeling as described previously [45] using the Random Primers DNA Labeling System kit (Life Technologies, Grand Island, NY). The GR, RC3 and GAP-43 probes were generated by PCR using primers specific for the glucocorticoid receptor cDNA (5′-TGCAGCAGTGAAATGGGCAA-3’; 5′-GGGAATTCAATACTCATGGTC-3’), RC3 cDNA (5′-AGCCAAGGACCCTCAACAC-3’; 5′-GAGGGGCTCACAAACACAGT-3’), and GAP-43 cDNA (5′-ACAAGGAAAAAGCTCAAAG-3’; 5′-GACGGGGAGTTATCAGTGG-3’). The generated GR, RC3 and GAP-43 probes were 533 bp, 356 bp and 300 bp, respectively. The plasmid containing the β-actin cDNA probe was kindly provided by Dr. Michael Prystowsky, Albert Einstein University. Filters were hybridized and washed as previously described [45]. Specific mRNA levels were normalized to the β-actin mRNA level of each sample.

Statistics

Data were analyzed by a two-way analysis of variance (ANOVA, factors: strain, treatment) with the exception of MWM, which was analyzed by 3-way ANOVA with repeated measures. The Bonferroni Multiple Comparisons test, with a $p < 0.05$ adjusted significance level, was

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

used when appropriate to identify significant differences between groups. In some situations, hypothesis testing using a Student's t-test was applied to determine the existence of significant differences between specific groups in the absence of significant strain-treatment interactions. All data are presented as mean + SEM.

Results

Hypo- or hyperthyroidism was confirmed in our animals by measuring plasma T4 levels at the beginning of the study (Table 1). Both treatments changed T4 levels significantly ($F[2,56] = 102.7$; $p<0.001$) from untreated controls, but no significant strain differences were observed. To confirm the presence of the previously characterized hypothyroxinemia in the $TR\alpha^{o/o}$ mice, we carried out a planned comparison of T4 levels between untreated B6 and $TR\alpha^{o/o}$ mice. As expected, untreated $TR\alpha^{o/o}$ mice had significantly lower plasma T4 levels than untreated B6 mice (Table 1; $F[1,21]=28.3$; $p<0.001$).

$TR\alpha^{o/o}$ mice are hypoactive in the OFT and FST

The open field test is a well-established behavioral assay that can be used as a test of fear and anxiety (or emotionality) and exploratory behavior under stressful conditions. Two measures of anxiety were taken: latency to leave the center of the arena and time spent in the center *vs* the periphery. An anxious mouse may hesitate to go to the periphery and freeze for a short while, thus registering a higher latency to leave the center, and will spend less time moving in the center area of the arena. The total distance traveled by the animal is a measure of locomotor activity under the stress of novel environment.

In the OFT, there was a general strain difference with $TR\alpha^{o/o}$ mice showing a hypoactivity/increased anxiety profile compared to B6 mice. The increased anxiety profile did not seem to be related to the adult hypothyroxinemia of the $TR\alpha^{o/o}$ mice, since even frank hypothyroid state did not alter the general behavioral pattern of B6 mice. Specifically, $TR\alpha^{o/o}$ mice took significantly longer to leave the center of the open field (latency from center) compared to B6 mice regardless of treatment (Figure 1a; Strain: $F[1,67] = 8.90$, $p< 0.01$), a measure often identified with increased anxiety/depression-like behavior. In agreement with this finding, $TR\alpha^{o/o}$ mice were less active in the inner circle of the open field compared to B6 mice regardless of thyroid status (inner distance; Figure 1c; Strain: $F[1,67] = 1$, $p< 0.001$). $TR\alpha^{o/o}$ mice also showed decreased locomotion, and hypothyroidism, induced by PTU diet, reduced total distance traveled in both strains (total distance; Figure 1b; Strain: $F[1,67] = 15.22$, $p< 0.001$; Treatment: $F[2,67] = 3.51$, $p< 0.05$).

The mouse forced swimming test (FST) [43] is a useful model to predict antidepressant activity [9]. Genetic factors, such as strain, contribute to the behavioral performance of mice in this model of depression. $TR\alpha^{o/o}$ mice tended to float more (greater immobility) than B6 mice, although PTU treatment reversed this trend: hypothyroidism increased floating (immobility) in the B6, but decreased it in the $TR\alpha^{o/o}$ mice (Figure 2; Strain: $F[1,65]= 1.8$, $p= 0.069$; Strain x treatment; $F[2,65] = 6.2$, $p< 0.01$).

Impaired learning and increased thigmotaxis in $TR\alpha^{o/o}$ mice

Since we were interested in spatial learning and memory in the $TR\alpha^{o/o}$ mice under conditions that are similar to those employed in the previous two tests, namely novel environment, we only employed the hidden platform version of this task. In the novel environment of the Morris water maze, $TR\alpha^{o/o}$ and B6 mice did not differ in their latency to locate the submerged platform on day 1 indicating no motor or visual impairments between these strains regardless of treatment. However, on day 2, $TR\alpha^{o/o}$ mice took significantly longer to find the hidden platform of the MWM than B6 mice, and PTU treatment further increased this learning deficit (Figure

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

3a; Strain: $F[1,67] = 8.93$, $p < 0.01$; Treatment: $F[2,67] = 8.28$, $p < 0.001$). Since the strain x treatment interaction only approached significance ($F[1,67]=2.7$, $p=0.076$), we also used a planned comparison of the hypothyroid mice of the different strains. When compared using a Student t-test, the $TR\alpha^{o/o}$+PTU animals were found to take significantly more time to locate the platform than the $TR\alpha^{o/o}$ mice on day 2 (Bins 5–8), while PTU treatment had no effect on the learning behavior of the B6 mice (Figure 3a).

Similar to the latency to platform measure, there were no significant differences across groups in thigmotaxis on day 1. On day 2, thigmotaxis (Figure 3b) was significantly greater in the $TR\alpha^{o/o}$ compared to the B6 mice regardless of treatment (day 2, Strain: $F[1,67]= 15.09$, $p < 0.001$), suggesting that their inability to locate the platform was not related to decreased activity, but rather to increased anxiety. Again, similar to the latency to platform learning measure, only the PTU-treated $TR\alpha^{o/o}$ mice showed increased thigmotaxis during the second day of training, while PTU treatment did not significantly affect performance of the B6 mice (Strain x treatment; $F[2,67]=5.77$, $p<0.01$). There were no significant differences in swim speed between groups.

In the probe trial, there were no significant strain differences in the number of platform crossings (Figure 3c). However, PTU treatment decreased their recall ability, particularly in the $TR\alpha^{o/o}$ mice as indicated by their decreased number of platform crossings (Strain x treatment; $F[2,67]=3.75$, $p<0.05$) compared to $TR\alpha^{o/o}$ controls. Thigmotaxis during the probe trial (Figure 3d) resembled the thigmotaxis during training: the hypothyroid $TR\alpha^{o/o}$ animals spent more time near the edge of the water maze than their euthyroid counterparts or the hypothyroid B6 mice (Strain x Treatment: $F[2,67] = 10.90$, $p < 0.001$).

Thus, $TR\alpha^{o/o}$ mice showed spatial learning and recall deficits in the MWM, which may be due to their increased anxiety-like behavior. The hypothyroid $TR\alpha^{o/o}$, but not the B6, mice showed an exaggerated deficit.

Differential Decreased Expression of GR, GAP-43 and RC3 in response to developmental and experimental hypothyroidism in $TR\alpha^{o/o}$ mice

Both GR and GAP-43 have been extensively studied and implicated in learning, and RC3 is positively regulated by thyroid hormones in the rodent brain [23,36]. Therefore, we determined the expression of GR, GAP-43 and RC3 in the amygdala, frontal cortex and hippocampus of our euthyroid, hypothyroid and hyperthyroid B6 and $TR\alpha^{o/o}$ mice. These three brain regions are involved in the behaviors tested in this study [7,8,26,30].

Alterations in GR, GAP-43 and RC3 mRNA levels were found in the amygdala, frontal cortex and hippocampus of $TR\alpha^{o/o}$ mice (Table 2). GR, RC3 and GAP-43 expressions were significantly decreased in the hippocampus of $TR\alpha^{o/o}$ mice compared to B6 mice (Strain: GR: $F[1,30]=7.8$; $p<0.05$; RC3: $F[1,30]=14.5$; $p<0.01$; GAP-43: $F[1,30]=7.26$; $p<0.01$). In addition to the hippocampus, GAP-43 and GR expression were also significantly decreased in the amygdala of $TR\alpha^{o/o}$ mice compared to B6 regardless of treatment (Strain: GAP-43: $F[1,30] =14.2$; $p<0.001$; GR: $F[1,30]=52.8$, $p<0.001$). Moreover, GR mRNA levels were significantly lower in the frontal cortex of $TR\alpha^{o/o}$ mice compared to B6 (Strain: $F[1,30]=47.9$, $p<0.001$).

Thyroxine administration had no effect on the expression of these genes, but PTU treatment had strain-dependent effects (Table 2). In the $TR\alpha^{o/o}$ mice, hypothyroidism increased hippocampal GAP-43 mRNA and RC3 mRNA levels (GAP-43: Strain x treatment; $F[1,30] =13.6$; $p<0.001$; RC3: Strain x treatment; $F[1,30]=3.92$, $p<0.05$) and decreased frontal cortex RC3 expression (Strain x Treatment: $F[1,30]=5.1$, $p<0.05$;).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Discussion

The present study demonstrated that deletion of all TRα isoforms was coupled with increased behavioral inhibition and spatial learning and memory deficits. Most of these effects were exaggerated in hypothyroid $TR\alpha^{o/o}$ mice, suggesting that thyroid hormones can enhance behavior and increase spatial learning via TRβ receptor-mediated events.

$TR\alpha^{o/o}$ mice are hypothyroxinemic [31], and although there is no information on the thyroid hormonal profile of pregnant $TR\alpha^{+/o}$ mice, they might also show decreased T4 levels similar to TRα2 heterozygotes [51]. Since maternal hypothyroxinemia increases the risk of neurodevelopmental deficits in the fetus [28,61], we chose to use C57BL/6J (B6) mice as controls instead of the wild type littermates of $TR\alpha^{o/o}$ mice, as customary. Thus, the potential cause of behavioral differences between the $TR\alpha^{o/o}$ and the B6 mice might include the residual original 129sv x B6 background of the $TR\alpha^{o/o}$ mice [16]. Alternatively, they may be caused by maternal hypothyroxinemia of the $TR\alpha^{o/o}$ mice and finally, but most importantly, by the lack of the TR alpha locus. Although the influence of 129sv background flanking genes, in addition to the targeted TRα ablation, cannot be excluded, the residual 129sv background is a less likely cause given that the $TR\alpha^{o/o}$ mice was backcrossed to B6 11–13 times by the time we received them (Dr. Samuel Refetoff, personal communication). Thus, the potential of the $TR\alpha^{o/+}$ mother being hypothyroxinemic outweighed the possibility of the residual 129sv background on the offspring.

A similar consideration could be made in other studies as well, where the heterozygote dam can affect the development of their wild type and homozygote fetuses differentially, depending on their genotypes. To our knowledge, this gene-prenatal environment interaction has not been considered in practice, quite the opposite; the value of identical maternal genotype has been purported regardless of the effects of fetal genotype-prenatal environment interactions.

Finally, the behavioral strain differences found could be due to the lack of all TRα isoforms leaving only the TRβ isoforms to mediate thyroid hormone effects. Earlier studies indicate no compensatory differences in the expression of TRβ in the periphery of $TR\alpha^{o/o}$ mice [31,49] but a region-specific compensatory over-expression of TRβ in the brain of $TR\alpha^{o/o}$ mice cannot be excluded. Regardless of the expression levels of TRβ, the behavioral consequences of TRα ablation could be due to lack of stimulation or repression of TRα-regulated genes or the activity of TRβ. The contribution of liganded TRβ on these behaviors could be ascertained by the effect of PTU treatment in $TR\alpha^{o/o}$ mice.

Behavioral consequences of the ablation of TRα, which are not exaggerated by hypothyroid milieu, may be due to the lack of stimulation or repression of TRα-regulated genes. These behaviors include the increased latency to leave the center and the decreased activity in the inner circle of the OFT, both suggesting increased fear and anxiety. Since previous studies found that $TR\alpha1^{-/-}$ mice show increased anxiety/fear-related behaviors in the OFT [19] and elevated plus maze [56], the lack of TR α1 might be responsible for the increased anxiety/fear shown by the $TR\alpha^{o/o}$ mice. On the other hand, overall locomotor activity is normal in $TR\alpha1^{-/-}$ mice [59], but moderately decreased in our $TR\alpha^{o/o}$ mice, indicating a role of TRα2 in locomotion.

Altered behaviors in the “euthyroid” $TR\alpha^{o/o}$ mice, which are worsened by their hypothyroidism, or are found only in the hypothyroid $TR\alpha^{o/o}$ mice, could be due to thyroid hormone effects mediated by TRβ. These include deficits in spatial learning and memory and increased immobility in the FST. Interestingly, impaired hippocampal-dependent learning is observed in the $TR\alpha1^{-/-}$ mice [19], but mice with TRβ deletion do not show deficits in the MWM [13,14]. This latter finding argues against the contribution of liganded TRβ to these behaviors. However, it is important to note we found increased anxiety/fear in both the learning

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

and memory components in TRα$^{o/o}$ mice, while no such differences were reported with TRβ deletion. TRα$^{o/o}$ mice demonstrated increased thigmotaxis, which is a manifestation of increased anxiety and fear. As the PTU-induced hypothyroid state further increased thigmotaxis in TRα$^{o/o}$ mice, liganded TRβ may regulate anxiety/fear in the MWM. Thus, our findings suggest that absence of TRα may affect transcription of TRβ-regulated genes involved in anxiety/fear, and in turn, affect TRα–regulated hippocampal functions, fundamental to spatial learning.

Both TRα and TRβ-regulated genes seem to influence immobility behavior in the FST. Euthyroid and particularly T4-treated TRα$^{o/o}$ mice demonstrated increased immobility compared to T4-treated B6 mice, while reducing thyroid hormone levels decreased immobility in TRα$^{o/o}$ mice only. These changes in response to thyroid hormonal milieu suggest that TRα-regulated genes may inhibit, while TRβ-regulated genes increase, immobility behavior.

Glucocorticoid receptor, GAP-43 and neurogranin (RC3) are potential neurochemical substrates of the behavioral changes observed in the TRα$^{o/o}$ mice. Immobility in the FST [6, 24], and learning and memory [38] are may be influenced by GR-mediated processes in the hippocampus. Thus, the novel finding of significantly decreased GR expression in the hippocampus (as well as amygdala and frontal cortex) of TRα$^{o/o}$ mice may explain their learning impairment. GAP-43 [60] and RC3 [17] regulate calmodulin availability in axons and dendritic spines, respectively, and are involved in learning and synaptic plasticity. Under neonatal hypothyroid conditions, brain RC3 expression is significantly reduced [23,36]. Overexpression of GAP-43 has been found to enhance learning [48], while downregulation of GAP-43 expression impairs learning [37]. Similarly, RC3 knockout mice demonstrate impaired spatial learning and memory [33] and increased anxiety/fear in the OFT [45]. Since these same behavioral abnormalities were found in the TRα$^{o/o}$ mice, decreased GAP-43 and RC3 expression may contribute to their behavioral impairments. Messenger RNA level differences are likely functional, since message and protein levels typically correlate for GR [4], as well as for GAP-43 [37,40] and RC3 [34].

Despite well-known TR-mediated actions of thyroid hormones in the brain, TR knockout mice surprisingly show no apparent abnormal brain development compared with neonatal hypothyroidism [18,22]. It has been proposed that the structural alterations found in hypothyroid animals may result from persistent transcriptional repression exerted by the unliganded TR [21,35]. Specifically, the lack of TRα may not only cause a loss of thyroid hormone responsiveness of target genes but could result in a broad increase in the expression of TRα-regulated genes due to the lack of constitutive silencing activity. In the TRα$^{o/o}$ mice, abrogation of the constitutive silencing mediated through TRα2, the ligand-unresponsive TR with a weak dominant negative effect [11,54], may alter the expression of TRα -mediated genes. Although we did not find any significant increases in GR, GAP-43 and RC3 in the TRα$^{o/o}$ mice in any of the brain regions studied, the possible effect of decreased constitutive silencing (mediated through TRα2) on these or other genes cannot be excluded.

Taken together these data demonstrate behavioral alterations in the TRα$^{o/o}$ mice, most of which are exaggerated by hypothyroid state. The strain differences in response to hypo- and hyperthyroidism in adulthood indicate the functional role of TRα, and a delicate interaction between TRα and TRβ-regulated genes in these behaviors. However, it is conceivable that the perinatal hypothyroidism of the TRα$^{o/o}$ mice is responsible for the behavioral abnormalities observed, due to insufficient thyroid hormone during CNS development, rather than the complete lack of the TRα gene. Further studies are required to distinguish the effect of perinatal hypothyroidism from that of TRα deficit on these behavioral measures, as well as on the expression of thyroid hormone-regulated genes, GR, RC3 and GAP-43, potentially responsible for the learning deficit of TRα$^{o/o}$ mice. Since the behaviors investigated in this study are all

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

stress-responsive, further examination of the potential cross-regulation between stress-induced anxiety/fear, thyroid function and learning and memory is warranted.

Acknowledgements

The authors would like to thank Dr. Peter Kopp for critically reviewing this manuscript.

References

1. Adams PM, Stein SA, Palnitkar M, Anthony A, Gerrity L, Shanklin DR. Evaluation and characterization of the hypothyroid hyt/hyt mouse. I: Somatic and behavioral studies. Neuroendocrinology 1989;49:138–143. [PubMed: 2725835]
2. Akaike M, Kato N, Ohno H, Kobayashi T. Hyperactivity and spatial maze learning impairment of adult rats with temporary neonatal hypothyroidism. Neurotoxicol Teratol 1991;13:317–322. [PubMed: 1886541]
3. Anthony A, Adams PM, Stein SA. The effects of congenital hypothyroidism using the hyt/hyt mouse on locomotor activity and learned behavior. Horm Behav 1993;27:418–433. [PubMed: 8225263]
4. Aronsson M, Fuxe K, Dong Y, Agnati LF, Okret S, Gustafsson JA. Localization of glucocorticoid receptor mRNA in the male rat brain by in situ hybridization. Proc Natl Acad Sci U S A 1988;85:9331–9335. [PubMed: 3194428]
5. Auso E, Lavado-Autric R, Cuevas E, Del Rey FE, Morreale De Escobar G, Berbel P. A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. Endocrinology 2004;145:4037–4047. [PubMed: 15087434]
6. Bachmann CG, Bilang-Bleuel A, De Carli S, Linthorst AC, Reul JM. The selective glucocorticoid receptor antagonist ORG 34116 decreases immobility time in the forced swim test and affects cAMP-responsive element-binding protein phosphorylation in rat brain. Neuroendocrinology 2005;81:129–136. [PubMed: 15970644]
7. Bast T, Zhang WN, Feldon J. Hyperactivity, decreased startle reactivity, and disrupted prepulse inhibition following disinhibition of the rat ventral hippocampus by the GABA(A) receptor antagonist picrotoxin. Psychopharmacology (Berl) 2001;156:225–233. [PubMed: 11549225]
8. Baxter MG, Murray EA. The amygdala and reward. Nat Rev Neurosci 2002;3:563–573. [PubMed: 12094212]
9. Borsini F, Meli A. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology (Berl) 1988;94:147–160. [PubMed: 3127840]
10. Burgos-Trinidad M, Koenig RJ. Dominant negative activity of thyroid hormone receptor variant alpha2 and interaction with nuclear corepressors. Mol Cell Endocrinol 1999;149:107–114. [PubMed: 10375023]
11. de Escobar GM, Obregon MJ, del Rey FE. Maternal thyroid hormones early in pregnancy and fetal brain development. Best Pract Res Clin Endocrinol Metab 2004;18:225–248. [PubMed: 15157838]
12. Forrest D. The developing brain and maternal thyroid hormone: finding the links. Endocrinology 2004;145:4034–4036. [PubMed: 15321949]
13. Forrest D, Golarai G, Connor J, Curran T. Genetic analysis of thyroid hormone receptors in development and disease. Recent Prog Horm Res 1996;51:1–22. [PubMed: 8701076]
14. Forrest D, Hanebuth E, Smeyne RJ, Everds N, Stewart CL, Wehner JM, Curran T. Recessive resistance to thyroid hormone in mice lacking thyroid hormone receptor beta: evidence for tissue-specific modulation of receptor function. Embo J 1996;15:3006–3015. [PubMed: 8670802]
15. Fraichard A, Chassande O, Plateroti M, Roux JP, Trouillas J, Dehay C, Legrand C, Gauthier K, Kedinger M, Malaval L, Rousset B, Samarut J. The T3R alpha gene encoding a thyroid hormone receptor is essential for post-natal development and thyroid hormone production. Embo J 1997;16:4412–4420. [PubMed: 9250685]
16. Gauthier K, Plateroti M, Harvey CB, Williams GR, Weiss RE, Refetoff S, Willott JF, Sundin V, Roux JP, Malaval L, Hara M, Samarut J, Chassande O. Genetic analysis reveals different functions for the products of the thyroid hormone receptor alpha locus. Mol Cell Biol 2001;21:4748–4760. [PubMed: 11416150]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

17. Gerendasy DD, Sutcliffe JG. RC3/neurogranin, a postsynaptic calpacitin for setting the response threshold to calcium influxes. Molecular Neurobiology 1997;15:131–163. [PubMed: 9396008]

18. Gothe S, Wang Z, Ng L, Kindblom JM, Barros AC, Ohlsson C, Vennstrom B, Forrest D. Mice devoid of all known thyroid hormone receptors are viable but exhibit disorders of the pituitary-thyroid axis, growth, and bone maturation. Genes Dev 1999;13:1329–1341. [PubMed: 10346821]

19. Guadano-Ferraz A, Benavides-Piccione R, Venero C, Lancha C, Vennstrom B, Sandi C, DeFelipe J, Bernal J. Lack of thyroid hormone receptor alpha1 is associated with selective alterations in behavior and hippocampal circuits. Mol Psychiatry 2003;8:30–38. [PubMed: 12556906]

20. Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, Faix JD, Klein RZ. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. New England Journal of Medicine 1999;341:549–555. [PubMed: 10451459]

21. Hashimoto K, Curty FH, Borges PP, Lee CE, Abel ED, Elmquist JK, Cohen RN, Wondisford FE. An unliganded thyroid hormone receptor causes severe neurological dysfunction. Proc Natl Acad Sci U S A 2001;98:3998–4003. [PubMed: 11274423]

22. Hsu JH, Brent GA. Thyroid Hormone Receptor Gene Knockouts. Trends Endocrinol Metab 1998:103–112.

23. Iniguez MA, Rodriguez-Pena A, Ibarrola N, Aguilera M, Munoz A, Bernal J. Thyroid hormone regulation of RC3, a brain-specific gene encoding a protein kinase-C substrate. Endocrinology 1993;133:467–473. [PubMed: 8344193]

24. Jefferys D, Copolov D, Irby D, Funder J. Behavioural effect of adrenalectomy: reversal by glucocorticoids or [D-Ala2,Met5]enkephalinamide. European Journal of Pharmacology 1983;92:99–103. [PubMed: 6628539]

25. Kulikov A, Torresani J, Jenningros R. Experimental hypothyroidism increases immobility in rats in the forced swim paradigm. Neuroscience Letters 1997;234:111–114. [PubMed: 9364510]

26. Lacroix L, Spinelli S, Heidbreder CA, Feldon J. Differential role of the medial and lateral prefrontal cortices in fear and anxiety. Behav Neurosci 2000;114:1119–1130. [PubMed: 11142644]

27. Lafranchi SH, Haddow JE, Hollowell JG. Is thyroid inadequacy during gestation a risk factor for adverse pregnancy and developmental outcomes? Thyroid 2005;15:60–71. [PubMed: 15687825]

28. Lavado-Autric R, Auso E, Garcia-Velasco JV, Arufe Mdel C, Escobar del Rey F, Berbel P, Morreale de Escobar G. Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. J Clin Invest 2003;111:1073–1082. [PubMed: 12671057]

29. Lazar MA, Hodin RA, Darling DS, Chin WW. Identification of a rat c-erbA alpha-related protein which binds deoxyribonucleic acid but does not bind thyroid hormone. Mol Endocrinol 1988;2:893–901. [PubMed: 2903438]

30. Lee I, Kesner RP. Time-dependent relationship between the dorsal hippocampus and the prefrontal cortex in spatial memory. J Neurosci 2003;23:1517–1523. [PubMed: 12598640]

31. Macchia PE, Takeuchi Y, Kawai T, Cua K, Gauthier K, Chassande O, Seo H, Hayashi Y, Samarut J, Murata Y, Weiss RE, Refetoff S. Increased sensitivity to thyroid hormone in mice with complete deficiency of thyroid hormone receptor alpha. Proc Natl Acad Sci U S A 2001;98:349–354. [PubMed: 11120878]

32. MacNabb C, O'Hare E, Cleary J, Georgopoulos AP. Congenital hypothyroidism impairs response alternation discrimination behavior. Brain Research 1999;847:231–239. [PubMed: 10575092]

33. Miyakawa T, Yared E, Pak JH, Huang FL, Huang KP, Crawley JN. Neurogranin null mutant mice display performance deficits on spatial learning tasks with anxiety related components. Hippocampus 2001;11:763–775. [PubMed: 11811671]

34. Mons N, Enderlin V, Jaffard R, Higueret P. Selective age-related changes in the PKC-sensitive, calmodulin-binding protein, neurogranin, in the mouse brain. J Neurochem 2001;79:859–867. [PubMed: 11723178]

35. Morte B, Manzano J, Scanlan T, Vennstrom B, Bernal J. Deletion of the thyroid hormone receptor alpha 1 prevents the structural alterations of the cerebellum induced by hypothyroidism. Proc Natl Acad Sci U S A 2002;99:3985–3989. [PubMed: 11891331]

36. Munoz A, Rodriguez-Pena A, Perez-Castillo A, Ferreiro B, Sutcliffe JG, Bernal J. Effects of neonatal hypothyroidism on rat brain gene expression. Mol Endocrinol 1991;5:273–280. [PubMed: 1710032]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

37. Namgung U, Routtenberg A. Transcriptional and post-transcriptional regulation of a brain growth protein: regional differentiation and regeneration induction of GAP-43. Eur J Neurosci 2000;12:3124–3136. [PubMed: 10998096]
38. Oitzl MS, Fluttert M, Sutanto W, de Kloet ER. Continuous blockade of brain glucocorticoid receptors facilitates spatial learning and memory in rats. European Journal of Neuroscience 1998;10:3759–3766. [PubMed: 9875354]
39. Oppenheimer JH, Schwartz HL. Molecular basis of thyroid hormone-dependent brain. Endocrine Reviews 1997;18:462–475. [PubMed: 9267760]
40. Pascale A, Gusev PA, Amadio M, Dottorini T, Govoni S, Alkon DL, Quattrone A. Increase of the RNA-binding protein HuD and posttranscriptional up-regulation of the GAP-43 gene during spatial memory. Proc Natl Acad Sci U S A 2004;101:1217–1222. [PubMed: 14745023]
41. Paxinos G, KBJ F. The mouse brain in stereotaxic coordinates. 2001
42. Pop VJ, Kuijpens JL, van Baar AL, Verkerk G, van Son MM, de Vijlder JJ, Vulsma T, Weirsinga WM, Drexhage HA, Vader HL. Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. Clinical Endocrinology 1999;50:159–155.
43. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for anitdepressants. Archives Internationales de Pharmacodynamie et de Therapie 1977;229:327–336. [PubMed: 596982]
44. Prange AJ Jr, Lara PP, Wilson IC, Alltop LB, Breese GR. Effects of thyrotropin-releasing hormone in depression. Lancet 1972;2:999–1002. [PubMed: 4116985]
45. Redei E, Halasz I, Li LF, Prystowsky MB, Aird F. Maternal adrenalectomy alters the immune and endocrine functions of fetal alcohol-exposed male offspring. Endocrinology 1993;133:452–460. [PubMed: 8344191]
46. Redei EE, Solberg LC, Kluczynski JM, Pare WP. Paradoxical hormonal and behavioral responses to hypothyroid and hyperthyroid states in the Wistar-Kyoto rat. Neuropsychopharmacology 2001;24:632–639. [PubMed: 11331143]
47. Rittenhouse PA, Redei E. Thyroxine administration prevents streptococcal cell wall-induced inflammatory responses. Endocrinology 1997;138:1434–1439. [PubMed: 9075699]
48. Routtenberg A, Cantallops I, Zaffuto S, Serrano P, Namgung U. Enhanced learning after genetic overexpression of a brain growth protein. Proc Natl Acad Sci U S A 2000;97:7657–7662. [PubMed: 10861025]
49. Sadow PM, Koo E, Chassande O, Gauthier K, Samarut J, Xu J, O'Malley BW, Seo H, Murata Y, Weiss RE. Thyroid hormone receptor-specific interactions with steroid receptor coactivator-1 in the pituitary. Mol Endocrinol 2003;17:882–894. [PubMed: 12576486]
50. Sala-Roca J, Marti-Carbonell MA, Garau A, Darbra S, Balada F. Effects of dysthyroidism in plus maze and social interaction tests. Pharmacol Biochem Behav 2002;72:643–650. [PubMed: 12175461]
51. Salto C, Kindblom JM, Johansson C, Wang Z, Gullberg H, Nordstrom K, Mansen A, Ohlsson C, Thoren P, Forrest D, Vennstrom B. Ablation of TRalpha2 and a concomitant overexpression of alpha1 yields a mixed hypo- and hyperthyroid phenotype in mice. Mol Endocrinol 2001;15:2115–2128. [PubMed: 11731613]
52. Sap J, Munoz A, Damm K, Goldberg Y, Ghysdael J, Leutz A, Beug H, Vennstrom B. The c-erb-A protein is a high-affinity receptor for thyroid hormone. Nature 1986;324:635–640. [PubMed: 2879242]
53. Slone JL, Redei EE. Maternal alcohol and adrenalectomy: asynchrony of stress response and forced swim behavior. Neurotoxicol Teratol 2002;24:173–178. [PubMed: 11943505]
54. Tagami T, Kopp P, Johnson W, Arseven OK, Jameson JL. The thyroid hormone receptor variant alpha2 is a weak antagonist because it is deficient in interactions with nuclear receptor corepressors. Endocrinology 1998;139:2535–2544. [PubMed: 9564869]
55. van Boxtel, m; Menheere, p; Bekers, O.; Hogervorst, E.; Jolles, J. Thyroid function, depressed mood, and cognitive performance in older individuals: the Masstricht Aging Study. Psychoneuroendocrinology. 2004

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

56. Vasudevan N, Vasudevan M, Brewer C, Brewer D, Vennstrom B, DW P. Distinct behavioral phenotypes in male mice lacking the thyroid hormone receptor (TR)α1 vs. the TRβ gene isoforms. Society for Neuroscience Abstract 2001:27.

57. Versloot PM, van der Heide D, Schroder-van der Elst JP, Boogerd L. Maternal thyroxine and 3,5,3′-tri-iodothyronine kinetics in near-term pregnant rats at two different levels of hypothyroidism. European Journal of Endocrinology 1998;138:113–119. [PubMed: 9461326]

58. Weinberger C, Thompson CC, Ong ES, Lebo R, Gruol DJ, Evans RM. The c-erb-A gene encodes a thyroid hormone receptor. Nature 1986;324:641–646. [PubMed: 2879243]

59. Wikstrom L, Johansson C, Salto C, Barlow C, Campos Barros A, Baas F, Forrest D, Thoren P, Vennstrom B. Abnormal heart rate and body temperature in mice lacking thyroid hormone receptor alpha 1. Embo J 1998;17:455–461. [PubMed: 9430637]

60. Young EA, Owen EH, Meiri KF, Wehner JM. Alterations in hippocampal GAP-43 phosphorylation and protein level following contextual fear conditioning. Brain Res 2000;860:95–103. [PubMed: 10727627]

61. Zoeller RT. Transplacental thyroxine and fetal brain development. J Clin Invest 2003;111:954–957. [PubMed: 12671044]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

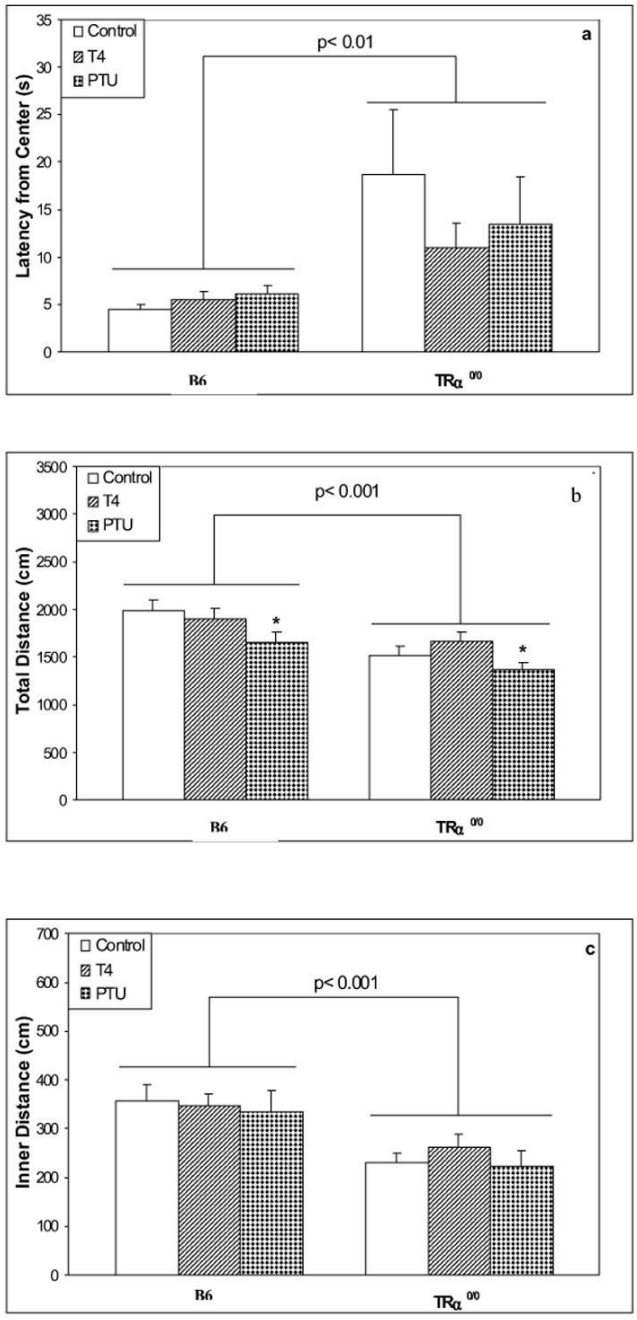


Figure 1.

Open Field Test Behavior. At 8 weeks of age, euthyroid (control), hyperthyroid (T4) and hypothyroid (PTU) C57BL/6J, the wild type (B6) and TRα$^{o/o}$ mice (n=9–13/group) were exposed to a novel open field. **(a)** After being placed in the center of the arena, a subject's latency to leave the center was measured in seconds (Latency from Center), **(b)** The total activity of the mice was measured by recording the total distance traveled in cm (Total Distance), **(c)** The activity of the mice in the inner region was measured by recording the distance traveled in the inner region in cm (Inner Distance). Values are means ± SEM. Asterisks indicate significant differences within a strain from the euthyroid control group (* = p< 0.05). Bars with a significance level above indicate significant strain differences.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

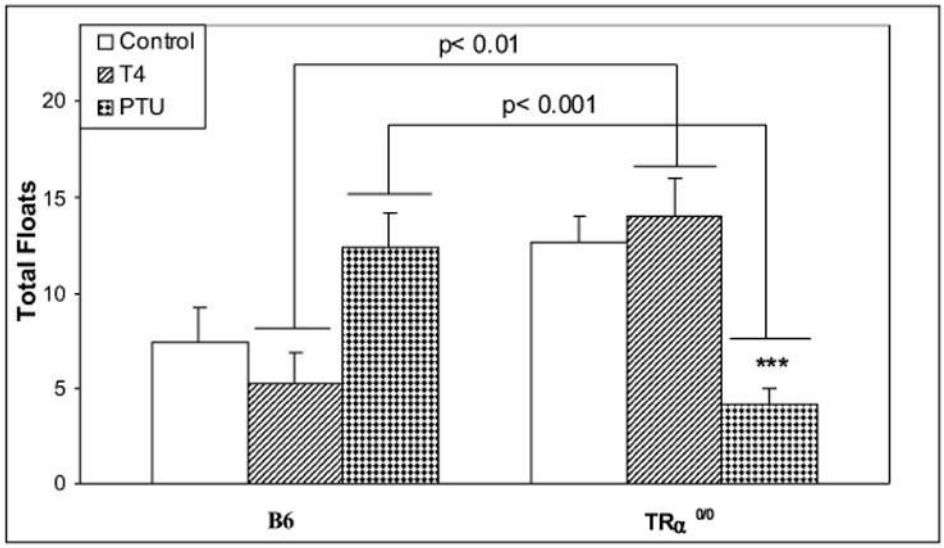


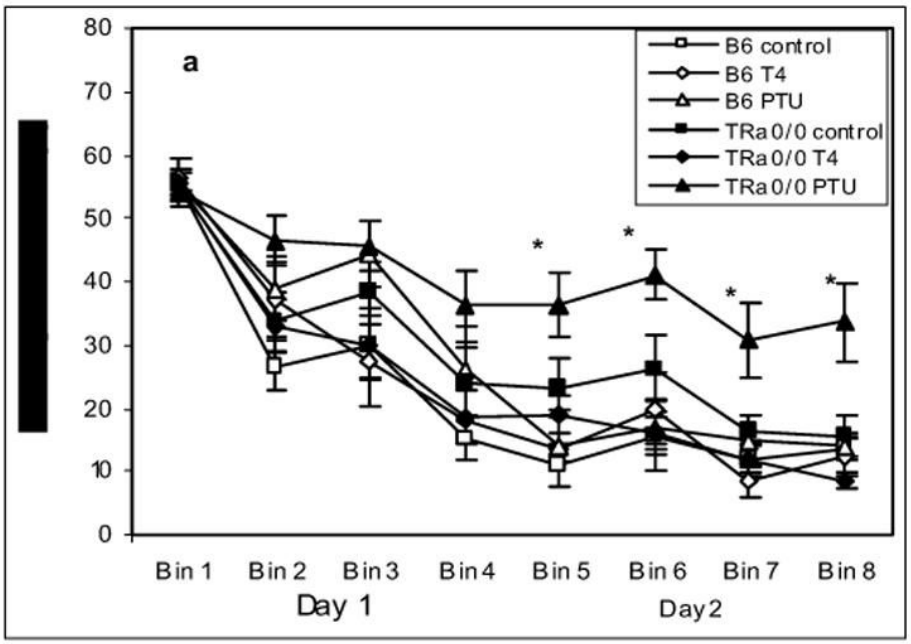
Figure 2.

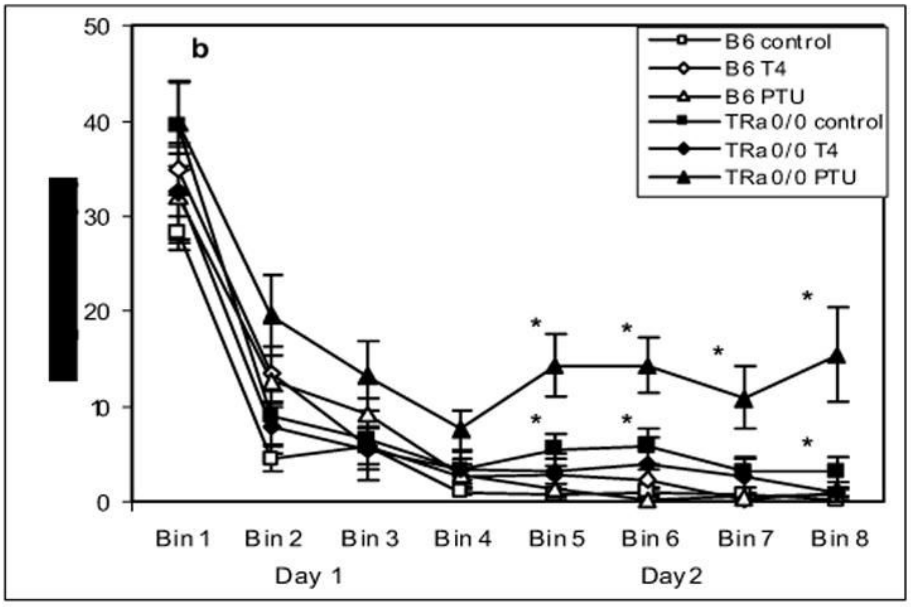
Forced Swim Test (FST). Between 13 and 15 weeks of age, mice (n=8–12/group) described in Figure 1 were subjected to the FST for 6 minutes. Behavior was recorded in five second bins using a time-sampling method. Values are means ± SEM. Asterisks indicate significant differences within a strain (*** = p< 0.001). Bars with a significance level above indicate significant differences between strains with the same treatment.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript





NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

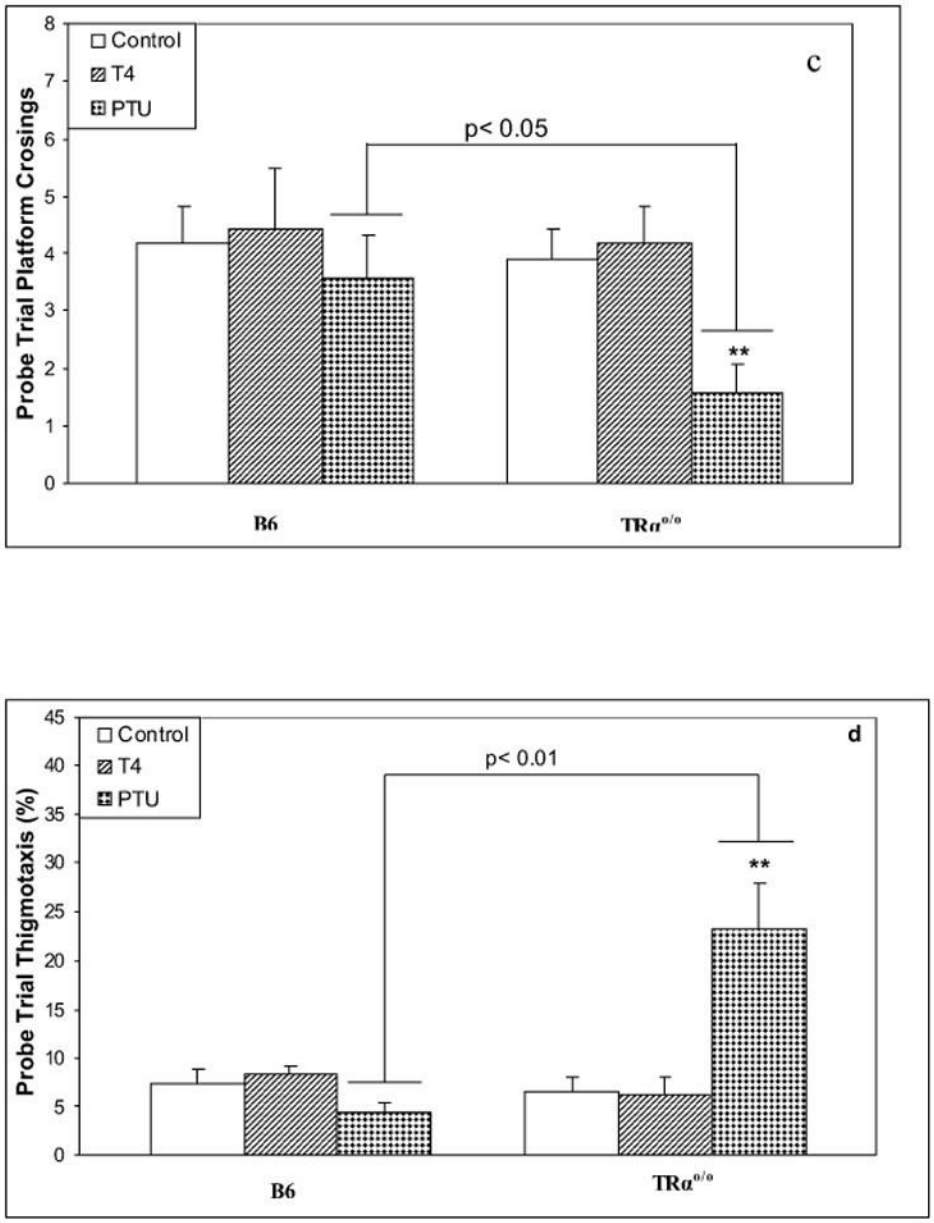


Figure 3.

Animals (10–12 weeks old, n=9–16/group) underwent training in the MWM to locate a hidden platform. Training consisted of 24 trials over two days. Trials occurred in bins: three one-minute trials conducted from the same starting location before moving the animal to the next starting location. **(a)** Latency for the animal to reach the platform was recorded. **(b)** Percentage of thigmotaxis (time spent near the edge of the pool) was chronicled during training. **(c)** Number of platform crossings in the quadrant of the hidden platform during the probe trial. **(d)** Percentage of thigmotaxis during the probe trial as measured. Values are means ± SEM. Asterisk indicates a significant ($p<0.05$) difference from groups with the same treatment but of different strains (a and b) or $p<0.01$ differences due to treatment within the strain (c and d). Bars with a significance level above indicate significant differences between strains with the same treatment (b and c).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 1

Plasma Thyroxine (T4) levels at the beginning of behavioral experiments.

Group	T4 (μg/dl)
B6	1.78+/−0.15
B6+T4	9.04+/−1.49*
B6+PTU	0.49+/−0.08*
TRα$^{o/o}$	0.79+/−0.07#
TRα$^{o/o}$+T4	10.09+/−0.7*
TRα$^{o/o}$+PTU	0.32+/−0.02*

Values are means +/− SEM of n=9–13 mice per group.

* p<0.05 from euthyroid mice of the same strain,

# p<0.05 strain difference.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 2

Messenger RNA levels of GR, GAP-43 and RC3 in amygdala, frontal cortex and hippocampus.

Group	GR	GAP-43	RC3
Amygdala			
B6	1.40 +/− .16	2.23+/− .23	1.44 +/− .20
B6+T4	0.93 +/− .27	2.11 +/− .32	1.45 +/− .23
B6+PTU	0.76 +/− .29	1.40 +/− .53	1.23 +/− .11
TRα$^{o/o}$	0.60 +/− .17$^{\#}$	1.57 +/− .12$^{\#}$	1.07 +/− .20
TRα$^{o/o}$+T4	0.87 +/− .38	1.20 +/− .18	1.40 +/− .18
TRα$^{o/o}$+PTU	0.74 +/− .26	0.84 +/− .15	1.11 +/− .21
Frontal Cortex			
B6	1.50 +/− .15	1.46 +/− .31	0.72 +/− .20
B6+T4	1.03 +/− .14	1.47 +/− .35	0.65 +/− .11
B6+PTU	0.64 +/− .18	1.79 +/− .33	0.56 +/− .13
TRα$^{o/o}$	0.29 +/− .15$^{\#}$	2.20 +/− .27	1.02 +/− .14
TRα$^{o/o}$+T4	0.42 +/− .20	1.68 +/− .34	0.70 +/− .13
TRα$^{o/o}$+PTU	0.39 +/− .16	1.67 +/− .36	0.51 +/− .08*
Hippocampus			
B6	2.83 +/− .50	1.45 +/− .24	2.13 +/− .36
B6+T4	2.29 +/− .41	1.13 +/− .21	2.34 +/− .32
B6+PTU	1.48 +/− .56	0.74 +/− .52	1.77 +/− .50
TRα$^{o/o}$	0.58 +/− .43$^{\#}$	0.47 +/− .22$^{\#}$	0.31 +/− .40$^{\#}$
TRα$^{o/o}$+T4	1.76 +/− .72	1.10 +/− .30	0.98 +/− .77
TRα$^{o/o}$+PTU	1.32 +/− .68	1.49 +/− .23*	1.77 +/− .30*

Specific mRNA levels were normalized to the β-actin mRNA level of each sample. Values are means ± SEM.

* $p<0.05$ from euthyroid mice of the same strain,

$^{\#}$ $p<0.05$ strain difference.