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ORIGINAL ARTICLE

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Age effect on thyroid hormone brain response in male mice

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Abstract

Purpose Thyroid hormones (TH) are important for brain development and central nervous system (CNS) function. Disturbances of thyroid function occur with higher prevalence in the ageing population and may negatively impact brain function.

Methods We investigated the age impact on behavior in young adult and old male mice (5 vs. 20 months) with chronic hypo- or hyper-thyroidism as well as in sham-treated controls. Expression of TH transporters and TH responsive genes was studied in CNS and pituitary by in situ hybridization and qRT-PCR, whereas TH serum concentrations were determined by immunoassay.

Results Serum TH levels were lower in old compared with young hyperthyroid mice, suggesting a milder hyperthyroid phenotype in the aged group. Likewise, elevated plus maze activity was reduced in old hyperthyroid animals. Under hypothyroid conditions, thyroxine serum concentrations did not differ in young and old mice. Both groups showed a comparable decline in activity and elevated anxiety levels. However, an attenuated increase in hypothalamic *thyrotropin releasing hormone* and pituitary *thyroid stimulating hormone* transcript expression was found in old hypothyroid mice. Brain expression of *monocarboxylate transporter 8* and *organic anion transporting polypeptide 1c1* was not affected by age or TH status.

Conclusions In summary, ageing attenuates neurological phenotypes in hyperthyroid but not hypothyroid mice, which fits with age effects on TH serum levels in the animals. In contrast no changes in TH transporter expression were found in aged mouse brains with hyper- or hypo-thyroid state.

Keywords Ageing · Male mice · Thyroid hormones · Hypothyroidism · Hyperthyroidism

Introduction

Thyroid hormones (TH) are important for brain development and maintenance of central nervous system (CNS) function [1, 2]. In the adult human brain, TH excess is associated with significantly impaired attention, concentration, and verbal memory [3], whereas patients with TH deficiency may show mental slowness and depression [4, 5]. The prevalence of hyper- and hypo-thyroidism increases with age; however, how TH excess and deficiency affect CNS function is still poorly understood in the elderly [6]. While in humans, assessment of TH impact on brain activity is limited to behavioral tests and functional imaging by PET-CT or MRI [7–10], animal models, that lack critical components for TH signaling, have enabled a closer inside into molecular TH action in the brain. For example, the presence of the specific TH transporters monocarboxylate transporter 8 (Mct8; Slc16a2) and organic anion transporting polypeptide 1c1 (Oatp1c1; Slco1c1) is required for the transport of TH into the CNS. Consequently, deficiency of both transporters in mice resulted in a hypothyroid CNS state with delayed cerebellar development, reduced

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myelination and pronounced locomotor abnormalities [11]. Other studies, focusing on deiodinases which convert T4 into biologically active T3 (iodothyronine deiodinase type 2, Dio2) or inactive reverse T3 (iodothyronine deiodinase type 3, Dio3), have shown that Dio2 knockout mice with a 50% reduced brain T3 content exhibit increased anxiety and abnormalities in motor function at the age of 6 months [12, 13]. In contrast, mice lacking Dio3 had elevated brain T3 content and displayed decreased anxiety and reduced depression-like behavior [14]. Likewise, mice with global or neuronal expression of a dominant negative TH receptor α1 showed impaired recognition memory [15] and increased anxiety [16]. These studies underline the relevance of brain TH homeostasis for behavior and cognitive function, but have not addressed aging as an important modifier of TH action.

In the present study, we investigated whether age impacts specific CNS phenotypes of hyper- and hypothyroidism, e.g., activity, anxiety, learning, memory, and motor function. For this purpose, we rendered naturally aged male mice (5 and 20 months) hypo- or hyper-thyroid for 7 weeks by drug treatment and studied their neurocognitive and motor function in comparison to sham-treated mice using different behavioral tests. To address possible changes in brain TH signaling, mRNA expression pattern of TH transporters and TH responsive gene as well as regulative genes of the hypothalamic–pituitary–thyroid axis (HPT-axis) were analyzed.

Methods

Animals and treatment

Male wild-type C57BL/6NTac mice (Taconic Europe A/S) aged 5 and 20 months ($n = 8$–12 animals/treatment/age) were studied. These two age groups are appropriate to study the different life stages of mature adult (5–7 months) and old (20–21 months) mice. To simplify the description of both cohorts the younger group was named "young" and the older group "old".

Animals were individually housed under temperature-(23 ± 1 °C) and light-controlled (inverse 12:12 h light–dark cycle) conditions. Three weeks before start of the treatment, mice were habituated to the inverse light–dark cycle and kept under these conditions until killed. All experiments were performed during the animal's active phase. Food and water were provided ad libitum. All animal experiments including handling of mice were performed by the same experimenters.

Chronic hyperthyroidism was induced by i.p. injection of T4 at a dose of 1 µg/g body weight (Sigma-Aldrich) every 48 h as previously described [17]. For induction of chronic hypothyroidism, animals were fed a low-iodine diet (LoI; TD.95007, Harlan Laboratories) and received drinking water supplemented with 0.02% methimazole (MMI, Sigma-Aldrich), 0.5% sodium perchlorate (ClO_4-, Sigma-Aldrich), and 0.3% saccharine as sweetener (Sigma-Aldrich). In addition, hypothyroid and control animals received i.p. injection of PBS every 48 h. Control and hyperthyroid animals were fed a control diet (TD.95007 with added potassium iodide (0.0012 g/kg): TD.97350).

After 7 weeks of treatment, animals were killed and blood harvested by right ventricular heart puncture and serum collected as previously described [17]. For tissue collection, mice were perfused with heparinized saline through a needle placed in the left ventricle. Brains were removed and quickly frozen in 2-methylbutane (Sigma-Aldrich) cooled on dry ice and stored at −80 °C until further processing.

Determination of thyroid hormone serum concentrations

Total T4 (TT4), free T4 (FT4), and free T3 (FT3) serum concentrations were measured as previously described [17] using commercial ELISA kits according to the manufacturer's instructions (DRG Instruments GmbH). Serum samples of 6–10 mice were quantified and used for statistical analysis.

In situ hybridization (ISH) histochemistry

Plasmids containing cDNA fragments corresponding to nt 902–1598 of mouse Hr (NM_021877.2), to nt 8–443 of mouse Mct8 (GenBank accession number AF045692), nt 698–1168 of mouse Oatp1c1 (NM_021471.1), to nt 1251–1876 of mouse thyrotropin releasing hormone (Trh) (NM_009426), and to nt 190–445 of mouse thyroid stimulating hormone (Tsh) (NM_009432) were linearized and used as template for the synthesis of radiolabeled and unlabeled riboprobes. Radiolabeled riboprobes specific for Hr, Tsh, Trh, Mct8, and Oatp1c1 were generated by in vitro transcription using [^{35}S]-Uridine 5′-triphosphate as labeled substrate (Hartmann Analytic). ISH was carried out as described elsewhere [18], ^{35}S-labeled riboprobes were diluted in hybridization buffer to a final concentration of 9×10^4 cpm/µl for Hr, 1.5×10^4 cpm/µl for Oatp1c1 and 1.9×10^4 cpm/µl for Mct8. Unlabeled Tsh riboprobe (f.c. 0.5 ng/µl) was added to the hybridization buffer containing radiolabeled Tsh riboprobe (f.c.: 1×10^4 cpm/µl) and the radiolabeled Trh riboprobe (f.c. 1×10^4 cpm/µl) was diluted with unlabeled Trh riboprobe (f.c. 0.5 ng/µl).

In brief, frozen 20 µm brain and 14 µm pituitary sections were air-dried, fixed in 4% phosphate-buffered PFA solution (pH 7.4) for 1 h at RT, rinsed with PBS, permeabilized with 0.4% phosphate-buffered Triton X-100 for 10 min, and then washed with PBS. Acetylation was carried out in 0.1 M triethanolamine (pH 8.0) containing 0.25% (v/v) acetic anhydride. After 10 min, sections were rinsed with PBS,

dehydrated with 50% and 70% ethanol, and air-dried. Hybridization mix containing the respective riboprobes diluted in hybridization buffer (50% formamide, 10% dextran sulfate, 0.6 M NaCl, 10 mM Tris-HCl (pH 7.5), 1 × Denhardt's solution, 100 µg/ml sonicated salmon sperm DNA, 1 mM EDTA-di-Na, and 0.5 mg/ml t-RNA and 10 mM dithiothreitol).

Hybridization was performed overnight at 58 °C. Slides were rinsed in 2 × standard saline citrate (0.3 M NaCl and 0.03 M sodium citrate, pH 7.0) and subsequently treated with ribonuclease A/T1 at 37 °C for 30 min. Additional washing steps for 20 min each were carried out in 1×, 0.5×, 0.2 × standard saline citrate at RT followed by incubation in 0.2 × standard saline citrate at 65 °C for 1 h. For detection of radioactive ISH signals, sections were dehydrated and then exposed to X-ray films (BioMax MR; Eastman Kodak Co.) for 24–48 h. Subsequently, sections were dipped in Kodak NTB nuclear emulsion (Kodak) and stored at 4 °C for 2.5 (Trh), 17 (Oatp1c1), 20 (Hr), or 30 (Mct8) days. Pituitary sections hybridized with Tsh-specific riboprobes were incubated with photoemulsion for 2 days (hypothyroid), 3 days (control), and 7 days (hyperthyroid), respectively. Autoradiograms were developed and then analyzed under darkfield illumination. For quantification of signals with ImageJ, background signal intensities were measured and subtracted from the specific signal intensities. Values were normalized to the analyzed area. Experiments carried out using the respective sense probes did not produce any ISH signals. For quantification of Trh, Mct8, Oatp1c1, and Hr-specific hybridization signals, autoradiograms of $n = 4$–5 animals per experimental group were used while Tsh ISH signals were quantified on pituitary sections derived from $n = 5$–10 mice per group.

Quantitative Real-time PCR

Total RNA from striatum and cortex was isolated using the RNeasy Kit (Qiagen, Germany). RNA was reverse transcribed into cDNA with SuperScriptIII and random hexamer primers (Life Technologies, Germany). Quantitative real-time PCR was performed as described previously [17] and further details are given in supplemental material.

Statistical analysis

Calculation and plotting was done with GraphPad Prism 7 software. Results are presented as mean ± standard deviation or standard error of the mean, as indicated. Unpaired Student's t test was applied for determination of age differences in ISH experiments only if exposure times differed between the treatment groups. All other comparisons were tested for significance by two-way ANOVA and Tukey's post hoc test. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{\#}p < 0.0001$ were considered statistically significant.

Results

Thyroid hormone serum concentrations

Five- and twenty-month-old wild-type male mice were rendered hypo- or hyper-thyroid by a 7 week ClO_4- or T4-treatment, respectively. Serum samples were collected for determining TT4, FT4, and FT3 concentrations. T4-treated animals showed an approximately three- to fourfold rise of TT4, three- to sixfold rise of FT4 and approximately two- to threefold rise of FT3 concentrations in line with overt hyperthyroidism (Fig. 1a–c). Of note, FT4 serum concentrations were more than twofold higher in young compared with old hyperthyroid mice (Fig. 1b). In comparison, ClO_4- treatment reduced TT4 concentrations below detection limit in both age groups (Fig. 1a), but did not affect FT4 or FT3 serum levels (Fig. 1b, c). In fact, a slight elevation in FT3 concentration in old compared with young hypothyroid mice was noted (Fig. 1c).

In situ hybridization analysis of hypothalamic Trh and pituitary Tsh expression

The impact of exogenic TH modulation on the activity of the HPT-axis was assessed by radioactive ISH histochemistry. *Trh* mRNA expression was analyzed in the hypothalamic paraventricular nucleus, where the TH-sensitive hypophysiotropic *Trh* expressing neurons reside, while *Tsh* transcript levels were studied in the anterior pituitary. As illustrated in Fig. 2a, induction of hyper- and hypo-thyroidism resulted in overall expected changes in ISH signal intensities including suppressed expression of *Trh* and *Tsh* upon TH treatment and increased *Trh* and *Tsh* expression under ClO_4- treatment (Fig. 2a–c). Significant differences between young and old animals could not be detected under hyperthyroid conditions as TH treatment resulted in strongly diminished *Trh* and *Tsh* expression in both age groups. Under hypothyroid conditions, however, old animals exhibited significantly lower *Trh* and *Tsh* signal intensities compared with young mice. Of note, old control mice displayed higher *Trh* expression than young control mice while *Tsh* transcript levels were not different (Fig. 2b, c).

Thyroid hormone transporter expression in brain

Age-depending changes in brain TH homeostasis may occur due to an altered TH uptake into the CNS. As Mct8 and Oatp1c1 have been identified as critical transporters in mediating the TH passage into the murine brain, we included the analysis of *Mct8* and *Oatp1c1* mRNA expression patterns in our ISH study. As illustrated in Fig. 3a and in line with previous findings [19, 20], strong *Mct8*-specific hybridization signals were found in choroid plexus

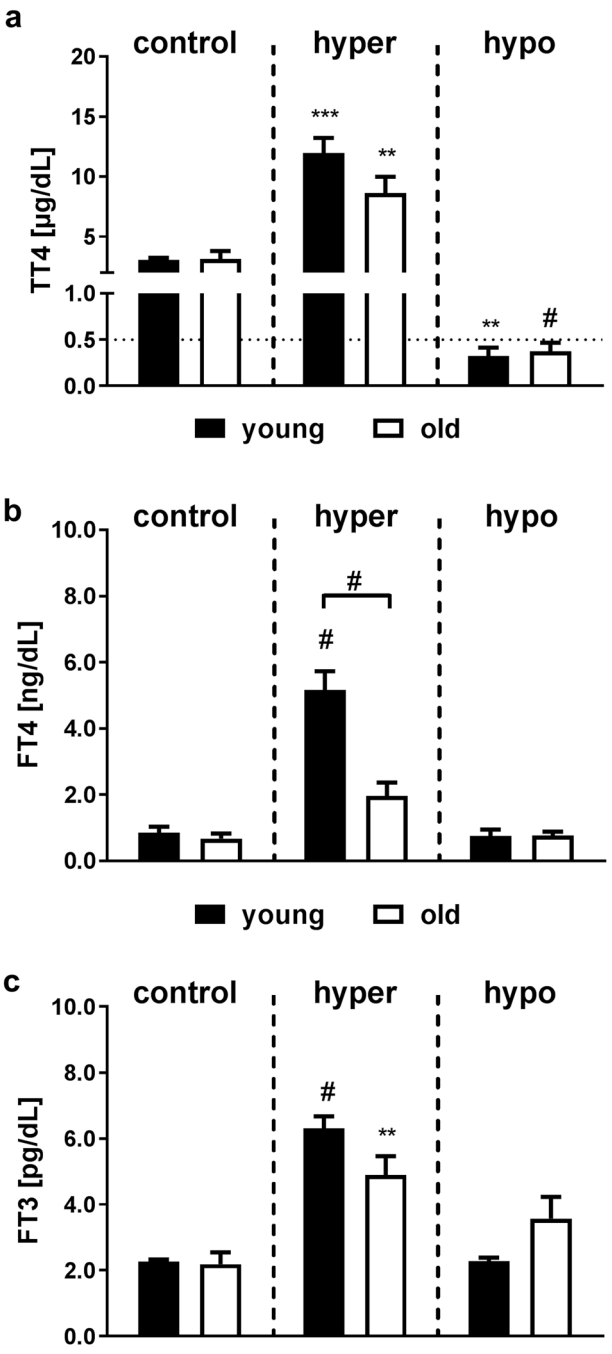


Fig. 1 TT4, FT4, and FT3 serum concentrations of young and old mice treated with T4 or LoI/MMI/ClO_4− for 7 weeks. Although T4 treatment caused elevated TT4 (**a**), FT4 (**b**), and FT3 (**c**) concentrations in both young and old males, old mice displayed a significantly lower increase in FT4. LoI/MMI/ClO_4− treatment resulted in strongly diminished TT4 concentrations while FT4 and FT3 values were not significantly affected by the treatment. Dotted line refers to detection limit of the TT4 assay and values below were calculated from standard curve. Mean ± SEM, age difference: symbols on half-tick down lines, TH-response: symbols over bars. ctrl control, hyper hyperthyroid, hypo hypothyroid

structures, in hypothalamic tanycytes, and in the hippocampal formation, while weaker signals were noted in the striatum and in the cerebral cortex. No obvious changes in *Mct8* signal intensities were noted between the different experimental groups. To confirm our visual impression, we quantified *Mct8*-specific hybridization signal intensities in the hippocampal dentate gyrus region, but could not detect any alterations between control, hypo- and hyper-thyroid animals. Only hyperthyroid old animals showed a slight but significant increase in *Mct8* transcript levels compared with hyperthyroid young animals (Fig. 3a, b).

Similar to *Mct8*, analysis of the *Oatp1c1* mRNA distribution pattern were in line with previous expression data and confirmed strongest hybridization signal intensities in choroid plexus structures and scattered *Oatp1c1*-specific signals throughout the brain (Fig. 3a, c). *Oatp1c1* mRNA expression was not significantly influenced by the age or the thyroid state of the animals (Fig. 3a, c).

Expression of TH responsive genes in the central nervous system

Hairless (*Hr*) is a well-established TH target gene that is frequently used to monitor the cellular TH status in brain [21]. We therefore included analysis of *Hr* mRNA expression in our ISH study as well. As expected and illustrated in Fig. 4a, *Hr*-specific ISH signal intensities were visibly upregulated under hyperthyroid condition and downregulated in hypothyroid animals. This visual impression could be substantiated by quantifying *Hr* signal intensities in the cerebral cortex (Fig. 4b). Moreover, hyperthyroid old animals showed higher *Hr* expression levels compared with young animals, thereby reflecting similar changes as found for *Mct8*.

To extend our analysis, we further quantified the transcript levels of TH-regulated genes by qRT-PCR using cortical and striatal areas. In particular we determined expression of *Ras homolog enriched in striatum (Rhes)* and *kruppel like factor 9* (*Klf9*) [22, 23]. However, for both genes, *Rhes* and *Klf9* expression we could not detect any changes under TH excess or deprivation (Fig. 5a–d). As expected, transcript levels of *Dio2*, known to be negatively regulated by TH, increased upon hypothyroidism in young and old animals in both investigated brain areas (Fig. 5e, f).

Locomotor activity in the elevated plus maze, motor function assessed by the rotarod test, and learning and memory by the Barnes Maze

To assess the impact of TH status and age on activity, motor function and learning capacity all animals were subjected to EPM, rotarod test and the Barnes Maze.

Locomotor activity was assessed by tracking the total distance of travel within the given time frame and revealed increased activity in young compared with old hyperthyroid mice. In contrast, hypothyroid animals showed reduced

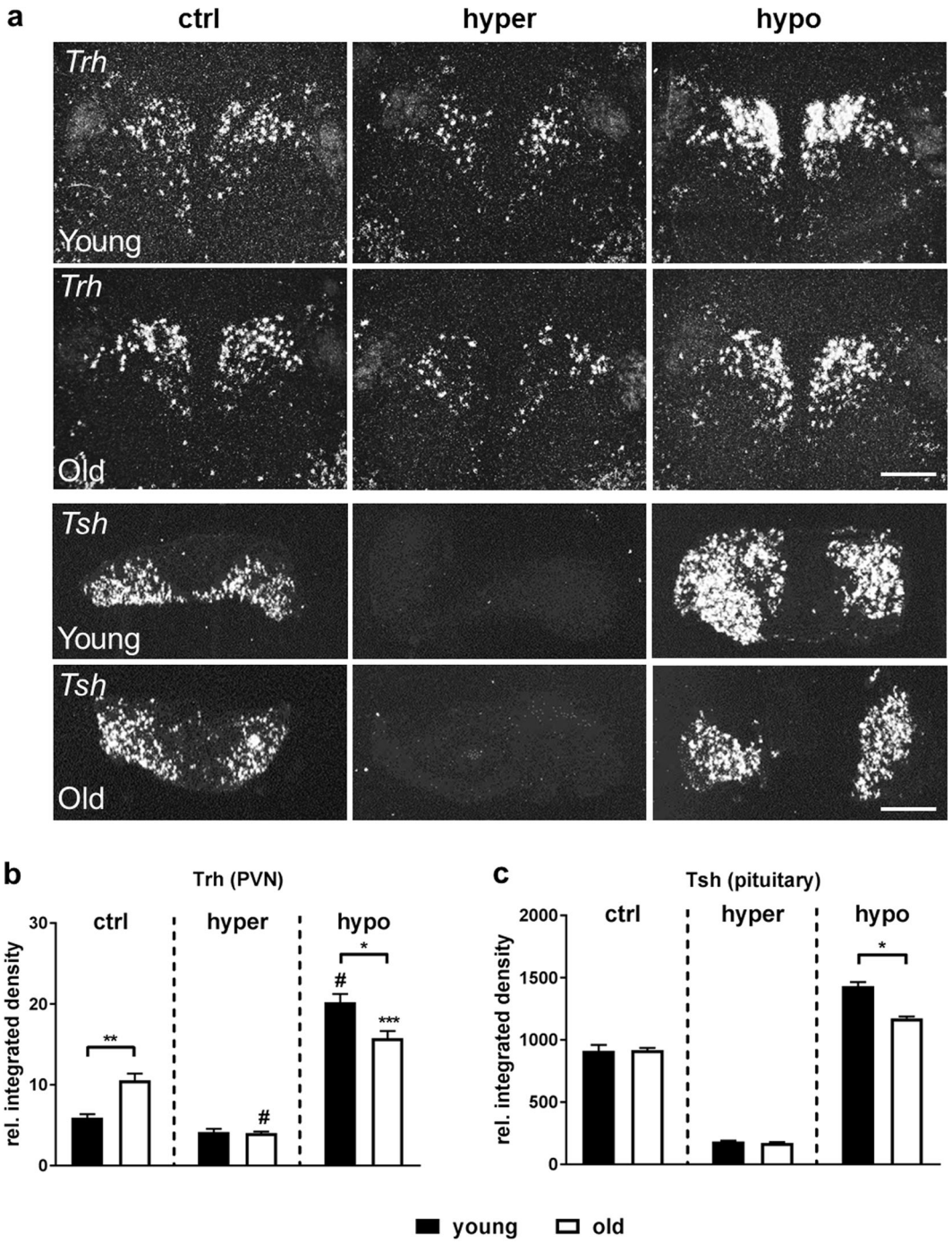
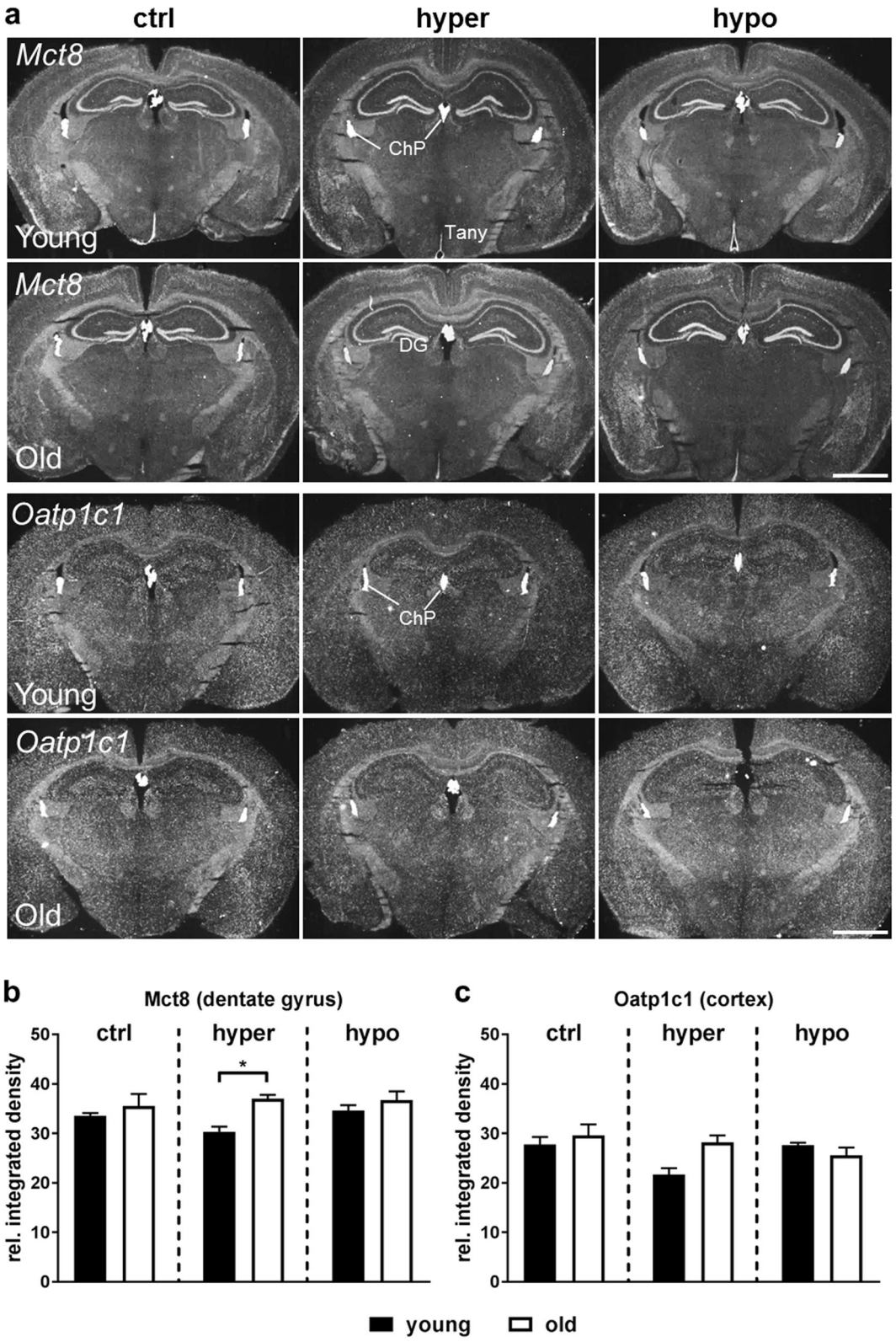


Fig. 2 Hypothalamic expression of *Trh* and pituitary *Tsh* expression in young and old mice under different TH conditions. Analysis of *Trh* and *Tsh* mRNA expression by ISH disclosed the expected changes in the hypothalamic paraventricular nucleus (PVN) for *Trh* and in the anterior pituitary for *Tsh* with decreased transcript levels in hyperthyroid animals and highly increased transcript levels in hypothyroid mice (**a**). The comparison of the ISH signal intensities between young and old animals revealed decreased *Trh* (**b**) and *Tsh* (**c**) mRNA levels in old mice under hypothyroid conditions. Moreover, old control animals showed elevated *Trh* but not *Tsh* levels compared with euthyroid control mice. Mean ± SEM, age difference: symbols on half-tick down lines, TH-response: symbols over bars. Scale bar indicates 200 µm for *Tsh* and 250 µm for *Trh*; ctrl control, hyper hyperthyroid, hypo hypothyroid

activity, irrespective of their age (Fig. 6a, b). As a second parameter, total freezing time was determined as a measure for immobility and was found to be elevated in hypothyroid mice of both age groups (Fig. 6c).

To investigate motor function, all mice were subjected to the accelerating rotarod task. In general, young mice performed significantly better than old animals and spend more time on the rod (Fig. 6d). The thyroid state did not



significantly influence motor performance in young mice, but old hypothyroid mice stayed longer on the rod compared with their respective control group.

For analyzing spatial learning capacity and memory, the widely used Barnes maze test was applied. On 4 consecutive days (with three trials per day) mice were allowed

◀ **Fig. 3** Differential expression of TH transporters in young and old mouse brains under control, hyper- and hypo-thyroid conditions. The expression of *Mct8* (upper panel) and *Oatp1c1* (lower panel) was analyzed in murine brain sections by ISH (**a**). Specific *Mct8* hybridization signals were identified in choroid plexus structures (ChP), hypothalamic tanycytes (Tany), and in the hippocampal formation. In comparison, strongest *Oatp1c1* expression was found in choroid plexus structures and specific signals were scattered throughout the brain. For a quantitative comparison, *Mct8*-specific hybridization signal intensities were determined in the hippocampal dentate gyrus region and revealed a significant increase in old hyperthyroid mice compared with young hyperthyroid animals (**b**). *Oatp1c1*-specific signals were quantified in the cerebral cortex and did not show any changes in relation to TH treatment or age (**c**). Mean ± SEM, age difference: symbols on half-tick down lines. Scale bar indicates 1 mm; ctrl control, hyper hyperthyroid, hypo hypothyroid, DG dentate gyrus

to find the escape box within 180 s (identified as spatial learning phase, Supplementary Fig. 1a), whereas on experimental day 5, short-term memory was tested and a single trial of max 90 s was conducted (Supplementary Fig. 1b). At day 12, this probe trial was repeated with no training in between in order to assess the long-term memory of mice (Supplementary Fig. 1c). In summary, hyper- and hypothyroidism had no significant effect on spatial learning, short- or long-term memory in mice of young and old mice in our study set-up.

Discussion

Decline in neurocognitive function is an essential issue in an ageing society and altered TH concentrations are suggested to promote this [2]. Many, if not all aspects of brain functioning including behavior, learning and memory are influenced by TH [1]. Our study aimed to investigate whether age impacts the neurological phenotype of hyper- or hypothyroidism in murine models and whether this includes changes in expression of TH transporters, TH deiodinases, and additional TH responsive genes in the CNS.

As a first step, however, we asked whether age had an impact on TH serum concentrations and the HPT-axis. Serum TH state defined by circulating TT4, FT4, and FT3 concentrations suggested less pronounced systemic hyperthyroidism in old mice. However, this had little impact on the HPT-axis as T3 responsive *Trh* and *Tsh* expression were fully suppressed in mice of both ages and therefore no differences could be noted. In contrast, mice rendered hypothyroid by LoI/MMI/ClO_4− treatment showed a pronounced drop in TT4 but no significant changes in FT4 levels compared with control animal. However, a rise in *Trh* and *Tsh* mRNA expression clearly pointed to a TH deficient situation in the hypothalamus-pituitary system. Of note, *Trh* and *Tsh* signals were higher in young compared with old animals suggesting local alterations in TH homeostasis. This supported our notion of slightly elevated FT3 serum concentrations in hypothyroid old mice, and suggested this difference to be relevant.

We therefore asked if TH transport into the brain could be altered. TH transporters, such as Mct8 and Oatp1c1, are mandatory for TH passage into the brain as mice deficient in both TH transporters exhibit a severe TH deficiency in the CNS [11]. Determination of *Mct8* and *Oatp1c1* expression pattern in the CNS of young and old mice under TH deficiency, excess and control conditions did not reveal any obvious changes in distribution pattern, indicating that the thyroid state does not significantly affect localization of TH transporter expression. These findings are in line with previous observations in athyroid $Pax8^{-/-}$ mice [19]. Furthermore, ageing had no effect on *Oatp1c1* expression, while *Mct8* expression was higher in brain sections of hyperthyroid old compared with young mice. However, as other studies have shown, that TH transporter expression drastically changes in early developmental stages of the brain and little at older age [20], it remains speculative whether our finding is physiologically relevant.

As a consequence of these findings, we expected few age-related effects on TH responsive gene expression in the brain. We addressed this hypothesis by expression analysis of the well-established TH responsive gene *Hr* in the brain, which increased upon TH excess and decreased under TH deprivation, as expected [21]. However, cortical *Hr* signal intensities were surprisingly stronger in old compared with young hyperthyroid mice, suggesting increased local T3 signaling in old mouse brains under TH excess. This hypothesis, however, could not be confirmed by including additional TH target genes in our analysis. Indeed, qPCR studies on the expression of *Rhes* and *Klf9* in cortex and striatum did not reveal any differences. This finding might be explained by the fact that some of the so-called TH target genes are only regulated by TH during distinct time windows. In comparison, an anticipated increased expression of *Dio2* expression could be detected under hypothyroid conditions.

Next, we asked to which extent activity of young and old mice changed under TH excess or deprivation and whether this may correlate with the observed changes in TH-dependent gene expression in the brain. TH deprivation is known to have a strong impact on activity of mice. Thus, we and others [24, 25] have observed a decrease in total activity and an increase in freezing time during the EPM trial in hypothyroid animals. This however did not change with age. The opposite effect was seen for TH excess, and here hyperactivity was observed for young mice only and correlated well with higher TH serum concentrations in these animals.

The impact of hyper- and hypo-thyroidism on neuromuscular function has been addressed in rodents in juvenile and developmental stages, but rarely in old mice. For

example, locomotor deficiencies were reported due to insufficient TH supply during fetal and perinatal development [26]. Furthermore, decreased muscle mass observed in mice devoid of both TR α and β demonstrated a strong impact of TH on muscle development, maintenance and function [27]. Similarly, mice lacking both major TH transporters Mct8 and Oatp1c1, showing a central hypothyroid but peripherally hyperthyroid phenotype, displayed severely reduced muscle performance and muscle strength [11].

We evaluated motor function by the rotarod task and found an improved performance in old hypothyroid compared with old control mice. This however could have been due to the lower body weight of mice in the hypothyroid group compared with respective controls (Supplementary Table 1). Overall, age had a major impact on motor function and all old animals spent decreased total time on the rod, irrespective of their TH status. In line with our findings, other studies reported voluntary exercise and muscle mass to decline with age in untreated C57BL6 male mice [28], which we confirm to be TH independent.

Since abnormal TH serum concentrations in hyper- and hypo-thyroidism are known to negatively affect spatial memory [29], the Barnes Maze task was conducted to evaluate possible differences in learning and memory of young and old mice under TH excess and deficiency. However, no age or treatment effect was noted for learning or memory in the Barnes Maze test, which might be less suitable experiment for assessment of cognitive function in mouse models of hyper- and hypo-thyroidism.

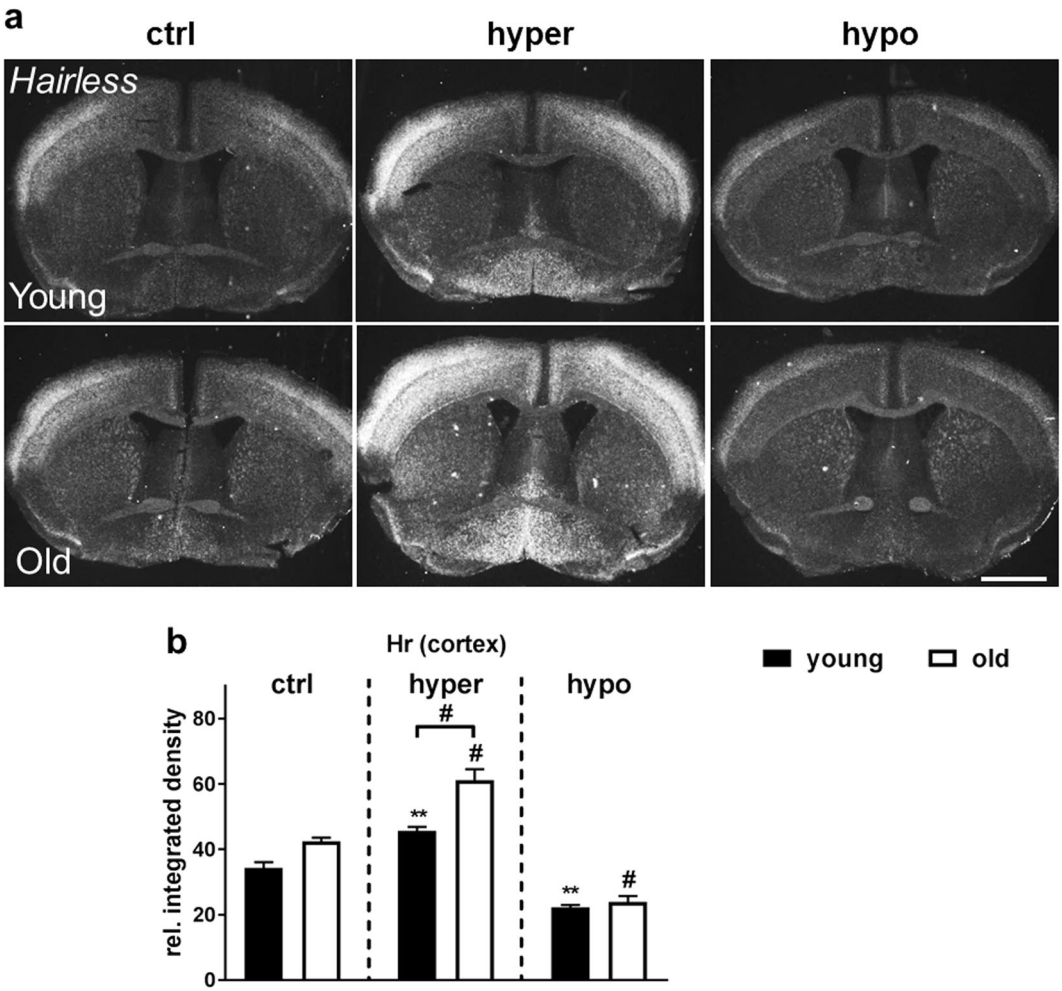


Fig. 4 mRNA distribution patterns of *Hr* in brains of young and old mice in control, hyper- and hypo-thyroid stages. Cryosections of mouse brains were hybridized with radioactively labeled riboprobes specific for the TH target gene *Hr* (**a**). Quantification of *Hr*-specific hybridization signals in the cerebral cortex revealed increased mRNA expression in hyperthyroid and reduced expression in hypothyroid animals. Of note, *Hr* signal intensities were significantly higher in old compared with young hyperthyroid mice. Mean ± SEM, age difference: symbols on half-tick down lines, TH-response: symbols over bars. Scale bar indicates 1 mm; ctrl control, hyper hyperthyroid, hypo hypothyroid

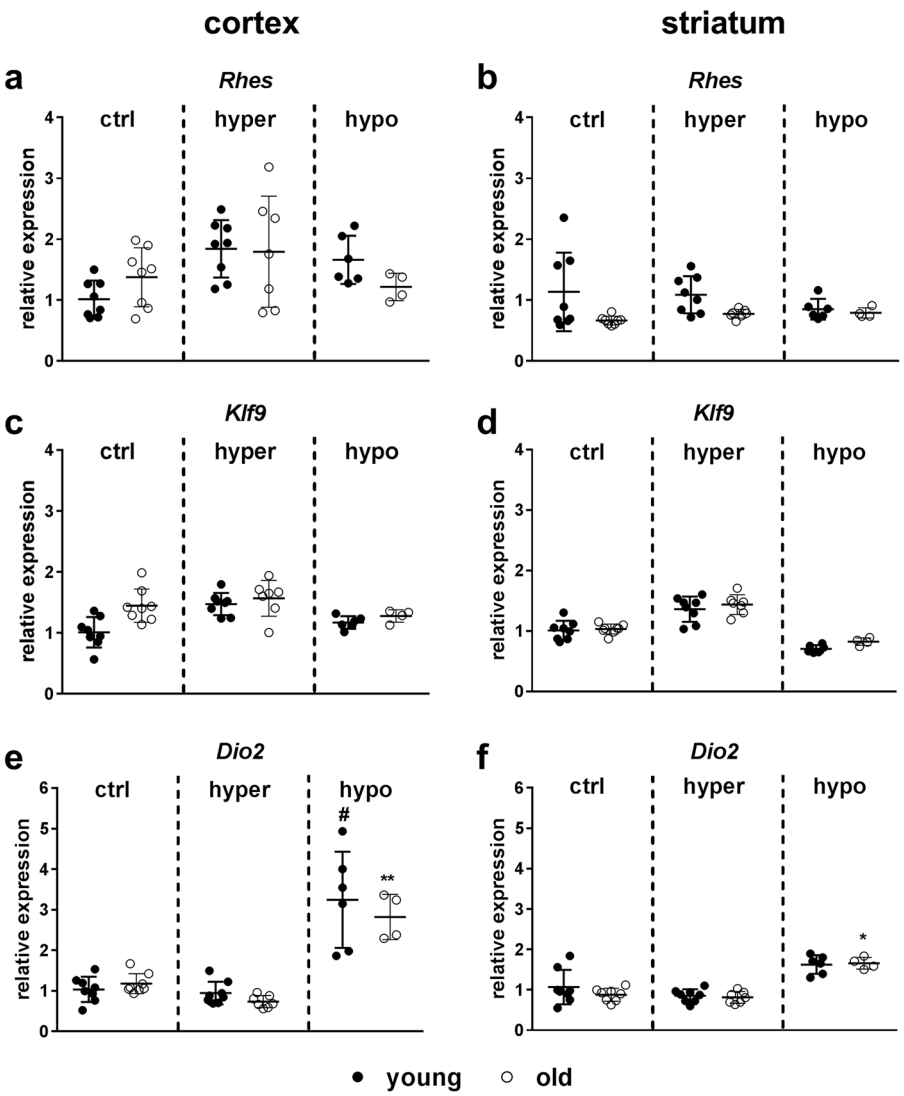


Fig. 5 qRT-PCR analysis of TH responsive genes and deiodinase type 2 in cortex and striatum of young and old mice. Gene expression analysis of TH target genes *Rhes and Klf9* was performed in cerebral cortex (**a**, **c**) and striatum (**b**, **d**), respectively, but did not reveal any changes. In contrast, *Dio2* mRNA expression increased upon hypothyroidism in cortex of young and old mice (**e**) and striatum of old mice (**f**). Individual values are presented as mean ± SD. ctrl control, hyper hyperthyroid, hypo hypothyroid

Conclusions

In our study, we found higher TH serum concentrations in hyperthyroid young compared with old mice, which did not directly correlate with *Hr* gene expression in cortex and did not induce changes in TH transporter and TH responsive gene *Rhes* and *Klf9* expression in the brain. However, altered TH concentrations affected activity, which increased in young mice only. In contrast a milder hypothyroid phenotype of old male mice was indicated by alteration in HPT-axis, but was not reflected in behavioral assessment, as a similiarly reduced EPM activity was found in both age groups.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All animal experiments were performed in accordance with the German regulations for Laboratory Animal Science (GVSOLAS) and the European Health Law of the Federation of Laboratory Animal Science Associations (FELASA). The protocols for animal studies were approved by the Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (LANUV-NRW), Germany (AZ.84–02.04.2013.A188).

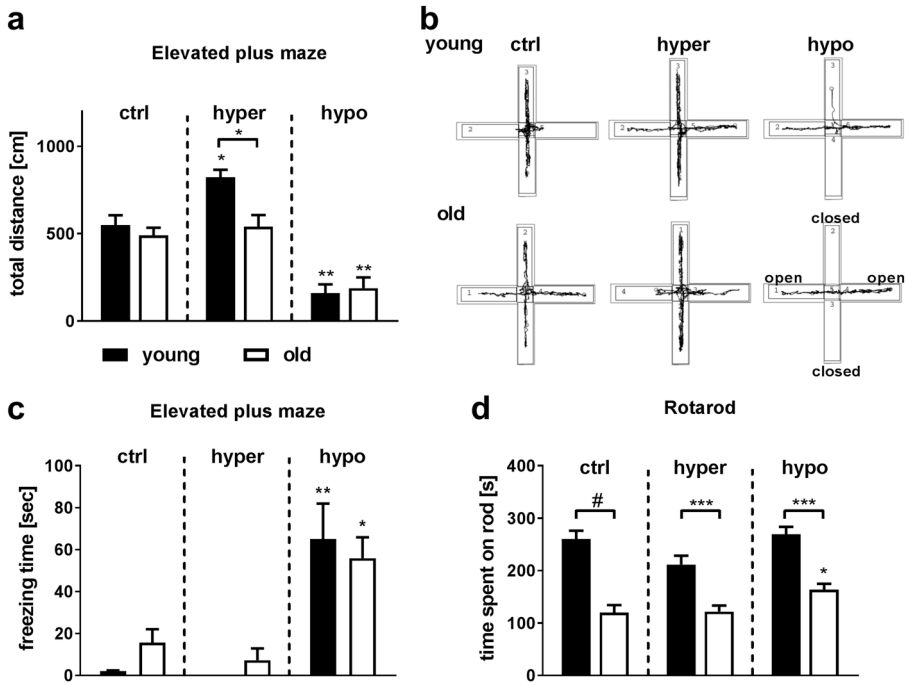

Fig. 6 Motor function in contrast to activity is age-dependent at all TH conditions. Locomotor activity and immobility of control-, hyper- and hypo-thyroid young and old mice were compared using an elevated plus maze test. For that purpose, the following parameters were quantified: the total distance the animals traveled (**a**) with respective exemplary tracking paths (**b**) and their freezing time (**c**). Increased activity was noted in young hyperthyroid mice, whereas hypothyroidism was associated with decreased activity and elevated immobility in both age groups. Motor function was assessed by rotarod testing (**d**). Independent of their TH status, young mice were able to stay significantly longer on the rotating wheel compared with old mice. Hypothyroid old mice in turn performed significantly better than control animals of the same age group. Mean ± SEM, age difference: symbols on half-tick down lines, TH-response: symbols over bars. ctrl control, hyper hyperthyroid, hypo hypothyroid

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