Allan-Herndon-Dudley Syndrome:
Pathogenic Mechanisms and Therapeutic Approaches

Inaugural Dissertation

for the attainment of the title of doctor
in the Faculty of Mathematics and Natural Sciences
at the Heinrich Heine University Düsseldorf

presented by

Eva Salveridou, M.Sc.

from Mönchengladbach

Düsseldorf, March 2020

This study was carried out from May 2016 to December 2017 at the Leibniz Research Institute for Environmental Medicine (IUF) in Duesseldorf, and from January 2018 to March 2020 at the department of Endocrinology, Diabetes and Metabolism at the University Duisburg-Essen under the guidance of Prof. Dr. Heike Heuer.

Published by permission of the
Faculty of Mathematics and Natural Sciences at
Heinrich Heine University Düsseldorf

Supervisor: Prof. Dr. Christine Rose

Co-supervisor: Prof. Dr. Ellen Fritsche

Date of the oral examination: 17.08.2020

I dedicate my PhD thesis to my wonderful family who always encouraged me.

Abstract

Patients with inactivating mutations in the thyroid hormone (TH) transporter MCT8 manifest a severe form of psychomotor retardation in combination with abnormal TH concentrations in the circulation (Allan-Herndon-Dudley Syndrome, AHDS). The neurological symptoms are most likely due to an impaired transport of TH into the central nervous system (CNS) and, consequently, a disturbed differentiation and maturation of neural cells. Passage of TH across the blood-brain barrier (BBB) and/or blood-cerebrospinal fluid barrier (BCSFB) is also impeded in mice lacking the TH transporters Mct8 and Oatp1c1. These Mct8/Oatp1c1 double knockout (M/O dko) mice display a delayed cerebellar development, diminished myelination and a disturbed maturation of GABAergic interneurons thereby replicating the abnormalities found in AHDS patients.

Treatment of AHDS patients with TH analogs that are not dependent on MCT8 but can activate TH receptors inside the brain has been suggested as a promising therapeutic approach. Candidates for such thyromimetic agents are the synthetic compound sobetirome (Sob1) and the recently developed four Sob derivatives (Sob2-5). The aim of this thesis was therefore to assess and to compare the thyromimetic potential of these novel compounds in neural development *in vitro* as well as *in vivo*.

In vitro studies were conducted using murine cerebellar cultures that were treated for two weeks with the TH receptor-active T3, Triac or with different concentrations of synthetic sobetirome compounds. Purkinje cell dendritic outgrowth was monitored and the number of PV+ interneurons was quantified, as both parameters are highly dependent on TH. While 10 nM Triac and 10 nM sobetirome were as effective as 1 nM T3 in promoting neuronal differentiation, significant lower doses (0.1 nM) of two sobetirome compounds (Sob2 and Sob5) were sufficient to achieve similar thyromimetic effects. Interestingly, both two prodrugs have to be first hydrolyzed into their active compound, a reaction that can be carried out by Fatty Acid Amide Hydrolase (FAAH). Indeed, application of FAAH specific inhibitors to primary cerebellar cultures abolished the Sob2- and Sob5- induced Purkinje cell development confirming a critical role of FAAH in prodrug-activation. Further, the capacity of sobetirome compounds to promote neuronal differentiation and myelination were also studied in M/O dko mice and revealed the strongest TH-like effects for Sob2 and Sob5. Thus, Sob2 and Sob5 may be considered as therapeutic options for patients with AHDS.

Zusammenfassung

Patienten mit inaktivierenden Mutationen im Schilddrüsenhormon (SDH) Transporter MCT8 leiden sowohl an einer schweren Form psychomotorischer Retardierung als auch an ungewöhnlichen SDH-Werten im Blut (Allan-Herndon-Dudley Syndrom, AHDS). Die neurologischen Symptome sind höchstwahrscheinlich auf einen eingeschränkten Transport der SDH ins zentrale Nervensystem (ZNS) und der daraus folgenden limitierten Differenzierung und Reifung der neuralen Zellen zurückzuführen. Der Transport der SDH über die Blut-Hirn-Schranke und/oder Blut-Liquor Schranke ist bei Mäusen, denen die beiden SDH-Transporter Mct8 und Oatp1c1 fehlen, ebenfalls beeinträchtigt. Diese Mct8/Oatp1c1 doppel knockout (M/O dko) Mäuse zeigen eine verzögerte Entwicklung des Kleinhirns, verminderte Myelinisierung und eine gestörte Reifung GABAerger Interneurone und spiegeln somit die Anomalien eines AHDS Patienten wider.

Ein vielversprechender Therapie-Ansatz für AHDS Patienten ist die Gabe von SDH-Analoga die unabhängig von MCT8 ins ZNS gelangen und dort SDH-Rezeptoren aktivieren können. Mögliche, thyromimetische Analoga sind das synthetisch hergestellte Sobetirome (Sob1), sowie die vor kurzem entwickelten vier Sob Derivate (Sob2-5). Somit war das Ziel dieser Arbeit das thyromimetische Potential dieser neuen Substanzen in der neuronalen Entwicklung *in vitro* und *in vivo* zu bestimmen und miteinander zu vergleichen.

In vitro Studien wurden anhand muriner, zerebellarer Kulturen durchgeführt, die für zwei Wochen mit dem SDH-aktiven T3, Triac oder unterschiedlichen Konzentrationen von Sobetiromen behandelt wurden. Dabei wurde das Auswachsen der Dendriten zerebellarer Purkinje Zellen analysiert, sowie die Anzahl an Parvalbumin positiven Interneuronen gemessen, da beide Parameter abhängig von SDH sind. Während 10 nM Triac und 10 nM Sobetirome ähnlich effektiv wie 1 nM T3 für die neuronale Differenzierung waren, konnten signifikant geringere Dosen (0.1 nM) für zwei der getesteten SDH-Analoga (Sob2 und Sob5) festgestellt werden, um ähnliche thyromimetische Effekte zu erzielen. Interessanterweise müssen diese beiden Prodrugs zunächst in ihre aktive Substanz hydrolisiert werden. Diese Reaktion kann durch die Fettsäureamid-Hydrolase ausgeführt werden. Eine Inhibierung dieses Enzyms durch Applikation spezifischer Inhibitoren in primärer, zerebellarer Kultur zeigte eine verminderte Sob2- und Sob5- induzierte Purkinje Zell Entwicklung und bestätigt somit die entscheidende Rolle der Fettsäureamid-Hydrolase für die Prodrug-Aktivierung. Des Weiteren, wurde die Fähigkeit der Sobetirome zur Förderung neuronaler Differenzierung, Myelinisierung und GABAerger Reaktionen im M/O Maus Modell untersucht. Die stärksten SDH-ähnlichen

Effekte zeigten Sob2 und Sob5. Somit können diese beiden SDH-Analoga als therapeutische Option für Patienten mit AHDS herangezogen werden.

Table of Contents

List of Abbreviations

AHDS	Allan-Herndon-Dudley Syndrome
BBB	blood-brain barrier
BCSFB	blood-cerebrospinal fluid barrier
BG	Bergmann glia
CB	calbindin
CC	corpus callosum
CNS	central nervous system
CSF	cerebrospinal fluid
D1/2/3	iodothyronine deiodinase type I/ II /III
DAPI	4′,6-diamidin-2-phenylindol
DIG	digoxigenin
Ditpa	3,5-diiodothyropionic acid
dko	double knockout
E	embryonic day
EGL	external granular layer
fl	floxed
GABA	gamma-aminobutyric acid
GAD	glutamic acid decarboxylase
GC	granule cell
HSM	horse serum medium
HPT axis	hypothalamus-pituitary-thyroid axis
IGL	internal granular layer
ISH	*in situ* hybridization
ko	knockout

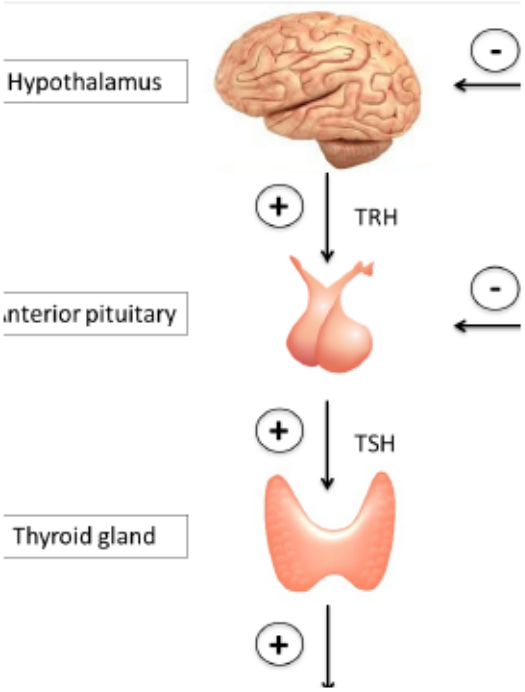
LAT	L-type amino acid transporter
MBP	myelin basic protein
MCT	monocarboxylate transporter
ML	molecular layer
NGS	normal goat serum
OATP	organic anion transporting polypeptide
OPC	oligodendrocyte precursor cell
P	postnatal day
PBS	phosphate-buffered saline
PC	Purkinje cell
PFA	paraformaldehyde
PV	parvalbumin
PVN	paraventricular hypothalamic nucleus
rT3	3,3′,5′-triiodothyronine; reverse T3
RTH	resistance to thyroid hormone
RXR	retinoid X receptors
SHBG	sex-hormone-binding globulin
T2	3,3′-diiodothyronine
T3	3,3′,5-triiodothyronine
T4	3,3′,5,5′-tetraiodothyronine, thyroxine
TH	thyroid hormone
TR	thyroid hormone receptor
Triac; TA3	3,3′,5-triiodothyroacetic acid
TRE	TH-responsive element
TRH	thyrotropin-releasing hormone
TSH	thyroid-stimulating hormone; thyrotropin
Wt	wild-type

1 Introduction

1.1 Thyroid hormones

Thyroid hormones (TH) are iodinated tyrosine derivatives produced and secreted by the thyroid gland. Two main forms are generated: 3,5,3´-triiodothyronine (T3) which is considered as the "active" TH based on its high affinity towards TH receptors, and 3,5,3´5´-tetraiodothyronine (thyroxin, T4), which is considered to function mainly as a prohormone for T3 (Gross and Pitt-Rivers, 1952; Gereben et al., 2008). The ratio of thyroidal secretion of T3 and T4 is species-dependent. While the rodent thyroid gland produces equal amounts of both hormones, a 4-fold lower secretion of T3 compared to T4 is observed in humans (Chen and Evans, 1995). More than 99% of the circulating TH are bound to plasma proteins, such as thyroxine-binding globulin (TBG), transthyretin (TTR) and serum albumin. Thereby, only free T3 and T4 can enter their target cells where they are metabolized and exert their biological functions (Chen and Evans, 1995; Krassas et al., 2007).

TH production is regulated by the hypothalamus-pituitary-thyroid (HPT) axis that determines the set point of TH synthesis and homeostasis by functioning in a classical endocrine negative feedback control mode (Fig.1.1). The HPT axis is regulated centrally by neurons located within the parvocellular region of the paraventricular nucleus (PVN) of the hypothalamus that synthesize and release the tripeptidamid thyrotropin-releasing hormone (TRH, pyroglutamate-histidine-proline-amide). Via axonal transport, TRH can reach the median eminence, which is connected with the anterior pituitary (adenohypophysis) by the hypothalamic-hypophysiotropic portal vein system (Lechan et al., 1994). Through binding to its TRH-receptor localized on thyrotrophs in the anterior lobe of the pituitary, TRH stimulates the synthesis and secretion of thyrotropin (thyroid-stimulating hormone, TSH) that in turn activates specific TSH-receptors at the surface of thyrocytes in the thyroid gland. Thereby, it mediates the subsequent activation of the intracellular second messenger cAMP (cyclic adenosine monophosphate) and protein kinaseA (PKA) signaling pathway that induces growth and differentiation of the thyroid gland and results in the stimulation of the synthesis and secretion of TH into the blood (Köhrle, 1990; Dremier et al., 2007; Wells and Murphy, 2003). The TH T4 and T3 control the secretion of TRH and TSH by negative feedback mechanism. Reduction of circulating TH levels results in increased TRH and TSH production, whereas the opposite occurs when circulating TH are in excess (Fekete and Lechan, 2007; Nikrodhanond et al., 2006).



(PVN) release thyrotropin-releasing hormone (TRH), which acts on pituitary thyrotropes to stimulat thyrotropin (TSH) synthesis. Following, TSH acts on thyrocytes to stimulate all steps of TH biosynthes in the thyroid gland. Subsequently, by a negative feedback regulation T3 and T4 reach PVN neurons a well as pituitary thyrotropes to inhibit TRH and TSH synthesis and secretion, respectively.

1.1.1 Metabolism of thyroid hormones

In humans, the thyroid gland produces predominantly the prohormone T4 (80%), whereas T3 only accounts for around 20% of TH synthesis (Wallis et al., 2008; Chanoine et al., 1993). TH activation and inactivation is achieved by enzymatic deiodination at different sites of the phenolic or tyrosyl ring of TH, mediated by the iodothyronine deiodinases (DIOs) D1, D2 and D3 (Bianco et al., 2002; Dentice et al., 2013). These enzymes are dimeric, membrane-anchored intracellular proteins with different expression patterns. They share substantial sequence homology and a highly active site containing the rare selenocysteine amino acid, which is critical for enzymatic activity. The majority of T3 in the circulation comes from deiodination of T4 at 5′ position of the outer ring (ORD). This step is catalyzed by D1 and D2 as depicted in Fig.1.2. Inactivation of T4 to rT3 and T3 to T2 is accomplished by D1 and D3, which exhibit inner ring deiodinase (IRD) activity. Abnormalities in deiodinase activity can alter local and systemic TH metabolism and are therefore important in a number of clinical settings (Bianco and Kim, 2006).

D1 is positively regulated by TH and contributes significantly to the production of serum T3. In humans and rodents, D1 is mainly expressed in peripheral tissues such as liver, kidney, intestine and thyroid gland (Bianco et al., 2002).

In rats, profound D2 activities have been found in the pituitary, brown adipose tissue (BAT), gonads, uterus and brain, among other tissues. In the CNS, high levels of D2 mRNA were present in astrocytes and tanycytes, specialized ependymal cells, lining the walls of the third ventricle (Tu et al., 1997). D2 appears to be especially responsible for the local supply of the CNS with T3 rather than contributing to global changes in serum TH levels (Guadaño-Ferraz et al., 1999; Bernal 2005; Bianco et al., 2002). In this context, studies in adult rats showed that 80% of the T3 content derives from D2-mediated T4-deiodination in the brain (Crantz et al., 1982). Thus, D2 ko mice exhibited reduced T3 concentrations in several major regions of the brain compared to control animals, despite an elevated T4 status in the CNS and in the circulation (Galton et al, 2007). In humans, D2 mRNA is expressed mainly in the thyroid, heart, brain, spinal cord, skeletal muscle and placenta (Bianco et al., 2002). In contrast to D1, D2 is regulated negatively on the transcriptional level but even more so on posttranslational level by TH through an ubiquitinase/deubiquitinase mechanism (Burmeister et al., 1997). Consequently, during TH deprivation, D2 activity increases in astrocytes thereby providing T3 for the neighboring neurons that are not able to generate T3 themselves (Guadaño-Ferraz et al., 1997; Fekete and Lechan, 2007) .

Finally, in humans and rodents, D3 is expressed in adult brain, skin and at high levels in multiple fetal tissues as well as in the placenta and the uterus during pregnancy. Its major function appears to protect tissues from excessive TH concentration (Bianco et al., 2002; Hernandez, 2005). In case of hypothyroid conditions, the activity of D3 is reduced, while in a hyperthyroid state, D3 activity is increased (Bianco et al., 2002).

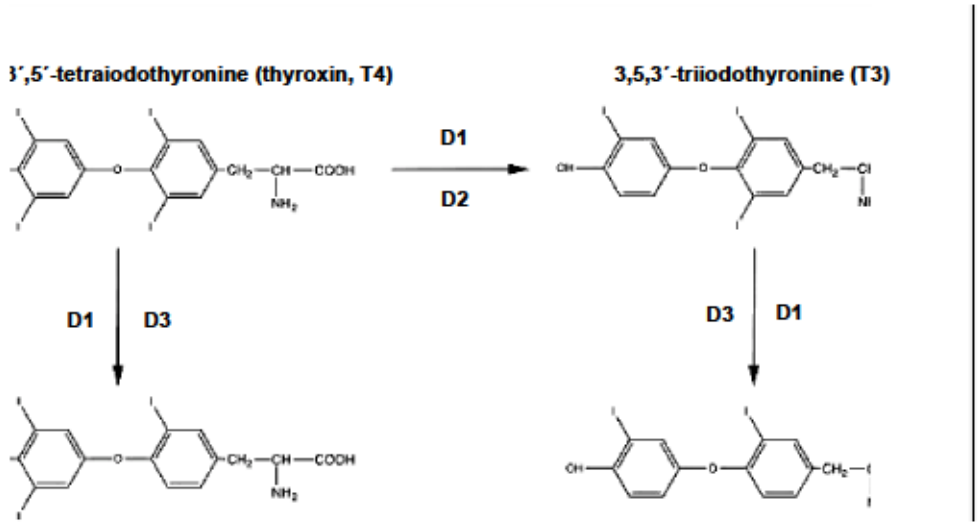


Fig.1.2 Overview of the metabolic activation and inactivation of TH by deiodination. Outer ring deiodination of the prohormone thyroxine (T4) into the biologically active form triiodothyronine (T3) is regulated by D1/D2. Contrarily, inner ring deiodination inactivates T4 into reverse-triodothyronine (rT3) and T3 into diiodothyronine (T2) by D1/D3 (modified from Aschner and Costa, 2015).

1.1.2 Thyroid hormone receptors

TH action is predominantly initiated by binding to nuclear TH receptors (TR) which function as ligand-modulated transcriptional factors. These receptors are constitutively bound to so-called TH responsive elements (TRE) in the promotor region of T3-sensitive target genes. They can form homodimers or heterodimers with retinoic acid receptors (RXR) (Brent et al.,1991; Yen et al., 2001).

T3-TR interaction modifies the organization of histones and chromatin as well as the recruitment of RNA polymerase II thereby causing the stimulation or the repression of gene expression in target cells (McKenna and O'Malley, 2002). Gene regulation by TR occurs both in the presence and in the absence of the ligand. Whereas T3-bound TR act as "holoreceptors" and stimulates genes that are positively regulated by TH, unliganded TR act as "aporeceptors" and suppress transcription of target genes by interacting with co-repressors such as NCoR (nuclear receptor co-repressor) and SMRT (silencing mediator of retinoic acid and thyroid hormone receptors). Binding of T3 leads to a conformational change in the TR, which releases co-repressors and recruits co-activators, such as nuclear receptor co-activator1 (NCoA-1 or SRC1) to induce gene transcription and mRNA production. In contrast, expression of genes that are negatively regulated by TH is stimulated by the aporeceptor- co-repressor complex and

repressed by the liganded TH-TR complexes. The exact underlying mechanism, is still poorly understood (Hörlein et al., 1995).

TR are expressed in a tissue- and cell-specific manner. They are encoded by two genes THRA and THRB, which are located on human chromosome 17 and 3 respectively. In mice, their homologues are Thrα and Thrβ (Brent, 2012; Flamant and Gauthier, 2013). Transcription of these genes generates a number of TR mRNA isoforms by alternative splicing. TRα1, TRα2 and TRα3 are products of THRA of which only TRα1 exerts DNA and T3-binding activity and can be therefore considered as a T3 receptor. TRα1 is already present at early developmental stages. Its highest expression level was found in heart, skeletal muscle and brain, where it was detectable in all neurons (Chassande et al., 1997; Wallis et al., 2010). TRα1 is involved in maintaining muscle strength, body temperature, energy expenditure and plays a crucial role in the early postnatal development and maturation of the brain (Johannsson et al., 2000; Flamant and Gauthier, 2013). TRa1 however, is of minor importance for the activity of the HPT axis, as deletion of TRα1 in mice leads only to mild hypothyroid state (Wikström et al., 1998). The potent aporeceptor activity was well described by the observation that transgenic mice lacking all TR have a relatively mild phenotype when compared with congenital hypothyroid mice: the receptor-deficient mice have a minor retardation in postnatal growth and survive until adulthood whereas the hypothyroid animals show major growth impairments and frequently die shortly after birth (Flamant et al., 2002; Göthe et al., 1999).

Mice carrying the dominant negative R384C point mutation in the TRα1 protein have a 10-fold reduced TH binding affinity for the receptor and exhibit a receptor-mediated hypothyroidism as well as a severe developmental delay (Tinnikov et al., 2002; Barca-Mayo et al., 2011). Using whole genome sequencing a heterogenous nonsense mutation was identified in the gene encoding for THRA that generates a mutant protein that inhibits Wt receptor action in a dominant negative manner and causes some target tissues to be resistant to the TH action (Bochukova et al., 2012; Mullem et al., 2013).

Growth retardation, delayed bone development, severe constipation and mild cognitive deficits are prominent symptoms in affected humans, while retarded brain development, growth retardation, anxiety and decreased glucose consumption were observations in mice with mutation in TRα1. Despite the fact that the clinical consequences of mutations in THRA are variable in extent and severity, thyroid function tests show an almost consistent pattern of normal TSH, low T4 and high T3 levels (van Gucht et al., 2017).

The THRB gene gives rise to two main products, TRβ1 and TRβ2 that both are able to bind T3 (Williams, 2000; Brent, 2012). TRβ1 is the major TRβ isoform and is detectable in liver, heart,

inner ear and retina. TRβ2 is highly expressed in the hypothalamus and pituitary, where it is considered to be the main isoform involved in the negative feedback mechanism of the HPT axis (Bradley et al., 1992; Lechan et al., 1994). A third isoform TRβ3 is found only in rats, where it is expressed in the kidney, liver and lungs (Williams, 2000). It has been reported, that mutations in the TRβ gene compromise the binding capacity of T3 and impair the negative feedback mechanism leading to increased TH levels with elevated TSH, T3 and T4 in the circulation. Patients affected by this TH resistance syndrome (RTHβ) caused by mutations in THRB show goiter formation, growth retardation and decreased body mass (Lazar, 1993). Similar changes were observed in mice that were completely deficient in TRβ. Further, homozygous Thrb deletion in mice caused auditory system defects and impaired vision (Forrest et al., 1996; Abel et al., 2001; Portella et al., 2010).

1.1.3 Thyroid hormones in brain development and function

TH are are essential for many physiological processes, such as development, growth, energy expenditure and metabolic homeostasis of almost all organs and tissues throughout life. Most importantly, they are crucially involved in the maturation and function of the CNS during fetal and postnatal period, regulating processes associated with brain differentiation, such as neuronal migration, axonal and dendritic growth, synaptogenesis and myelination. TH concentrations rise soon after birth and peak in the first postnatal years before declining again with age. TH deficiency in developing and adult human beings can lead to an extensive array of clinical manifestations, including neurological and psychiatric symptoms (Bernal, 2002; Anderson et al., 2003).

In the human fetus, the thyroid gland develops from the beginning of the second trimester. Before then, the only possible source of TH for the fetus is maternal origin. Untreated maternal hypothyroidism can lead to neurological cretinism, a disease characterized by severely stunted physical and mental growth with signs of neurological damage. Affected children eventually display deafness, perceptual motor problems, an inability to walk or speak and a clumsy movement (DeLong et al., 1985). Further, it has been shown that children born to mothers with hypothyroidism have an increased risk for attention-deficit hyperactivity disorder (ADHD) as well as autism spectrum disorder (ASD) (Vaidya, 2012; McPartland and Volkmar, 2012; Andersen et al., 2015) indicating that maternal TH exert critical function on fetal brain development.

Congenital hypothyroidism is characterized by a failure of endogenous TH production mainly caused by an impaired thyroid gland development that prenatally can be compensated by maternal TH supply. It is a relatively common disease, with an incidence of about 1 in 3000-4000 newborns (Delange, 1997). Affected children that are not treated with TH may suffer from mental and growth retardation as well but neonatal screening followed by immediate TH substitution therapy is usually sufficient to ensure normal postnatal development and brain maturation (Bernal, 2005; Thompson and Potter, 2000; Anderson et al., 2003).

Hypothyroidism may also develop later in life due to e.g autoimmune disease and has been associated with bipolar affective disorders, depression, or loss of cognitive functions. It can further cause lethargy, hyporeflexia, poor motor coordination and is often associated with memory impairment and personality changes (Bernal, 2000). The symptoms of hypothyroidism are usually reversible under proper TH treatment. Thus, TH alterations of adult onset do not cause permanent structural defects compared to fetal or early childhood TH deficiency (Bernal, 2000).

Most knowledge on TH action in the brain has been derived from studies of hypothyroid neonatal rats (Legrand, 1984). The rat fetus is provided with maternal TH through the placenta before the onset of endogenous TH production at embryonic day 17.5 (E17.5) (Anderson et al., 2003). After birth, TH serum levels rise until postnatal day 15 (P15) and decreases again during the development (Walker et al., 2012). These results indicate the importance of sufficient TH concentrations as well as timing of TH availability. Although the developmental events are similar among mammals, the timing signal for maturation programs during precise stages of the brain development presents substantial differences (Legrand, 1984; Bernal 2002). The rat brain at birth is more immature than the human brain at birth and rather reflects the situation of the developing human brain at six months gestation. The first postnatal week of a rat brain can be compared with a human brain around birth (Anderson et al., 2003; Legrand, 1984) (Fig.1.3).

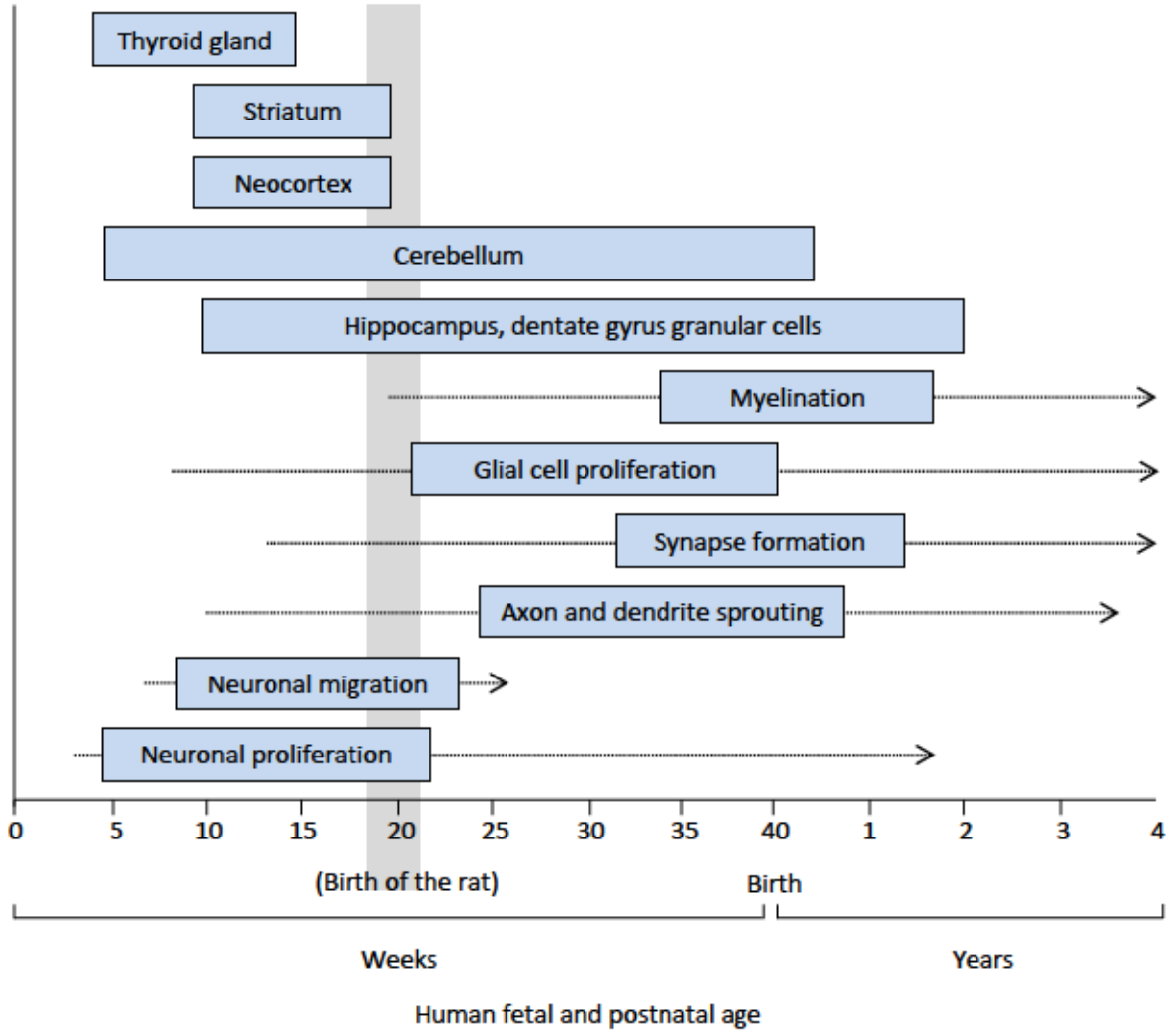


Fig.1.3 Timeline of the human brain development in relation to TH at fetal and postnatal age. For comparison with rat development, the equivalence for the birth of the rat is shown by a vertical grey line around postmenstrual week 20. Inappropriate initiation of these timing events leads to asynchrony in developmental processes with deleterious outcome (modified from Bernal, 2007; Hodeshell, 2002).

A vast number of studies showed the importance of TH action in different areas of the developing rat brain ranging from late fetal to postnatal stages and showed the consequences of hypothyroidism on a cellular level (Bernal, 2005; Anderson et al., 2003, Legrand, 1984). A prominent brain target of TH action is the cerebellum that in rodents develops mainly during the first three postnatal weeks. This brain structure is involved in balance, motor control and coordination and is depended on proper TH supply. The rodent cerebellar cortex has emerged as a preferred model to study TH action in the brain because of its sensitivity to T3 deficiency, its relatively simple cytoarchitecture compared with other brain areas and its precisely reported subsets of neurons and their developmental progression (Bernal, 2007; Koibuchi and Chin, 2000). The cerebellum has a three-layered laminar structure: an outer molecular layer (ML) containing basket and stellate cells, a middle Purkinje cell layer (PCL) containing Purkinje cells

(PC) and Bergmann glial (BG) fibers and an inner granule layer (IGL) containing granule cells (Fig.1.4A). During the first postnatal week, a fourth layer, the external granular layer (EGL) is present and populated by proliferating granule cell (GC) precursors, which randomly exit the cell cycle and undergo radial migration towards the IGL along the radial fibers of the BG, to ultimately differentiate into GC. At the same time, GABAergic interneuron progenitors and oligodendrocyte precursor cells (OPC) migrate in the opposite direction from the white matter and γ-aminobutyric acid (GABA)ergic interneurons undergo terminal differentiation in the molecular layer to become stellate or basket cells (Chédotal, 2010; Yamanaka et al., 2004).

TH deficiency in the cerebellum has been shown to induce various anatomical alterations (Fig.1.4B). These include: a) alteration of the development of BG radial fibers and the reduction of growth and branching of dendritic arborization of PC that reduces the thickness of the molecular layer; b) delay in synaptic formation between PC and GC axons; c) delayed proliferation and migration of GC that results in the persistence of EGL beyond postnatal day (P) 21 and impedes its migration towards IGL and the maturation of GC; d) delayed myelinations; e) changes in synaptic connection among cerebellar neurons and afferent fibers (Legrand et al., 1976; Legrand et al., 1984; Nicholson and Altman., 1972; Hajós et al., 1973; Lauder, 1977).

Previous studies detected all TR isoforms in the rodent brain with the highest expression of TRα1 (Ercan-Fang et al., 1996). In the cerebellum, TRα1 accounts for about 80% of T3 binding capacity and is found ubiquitously in PC, GC and BG, whereas TRβ1 is mainly found in PC.

Although TRβ1 is highly expressed during a critical time window of dendritic outgrowth and synaptogenesis it seems to be not essential for the dendritogenesis, since PC derived from TRβ1 ko mice showed the same T3 responsiveness as control cells (Strait et al., 1991; Bradley et al., 1992; Heuer and Mason, 2003).

TRα1 expression is upregulated in GC at the time when these cells undergo the last cell division and become prepared to migrate inwardly from EGL (Bradley et al., 1992; Wallis et al., 2010).

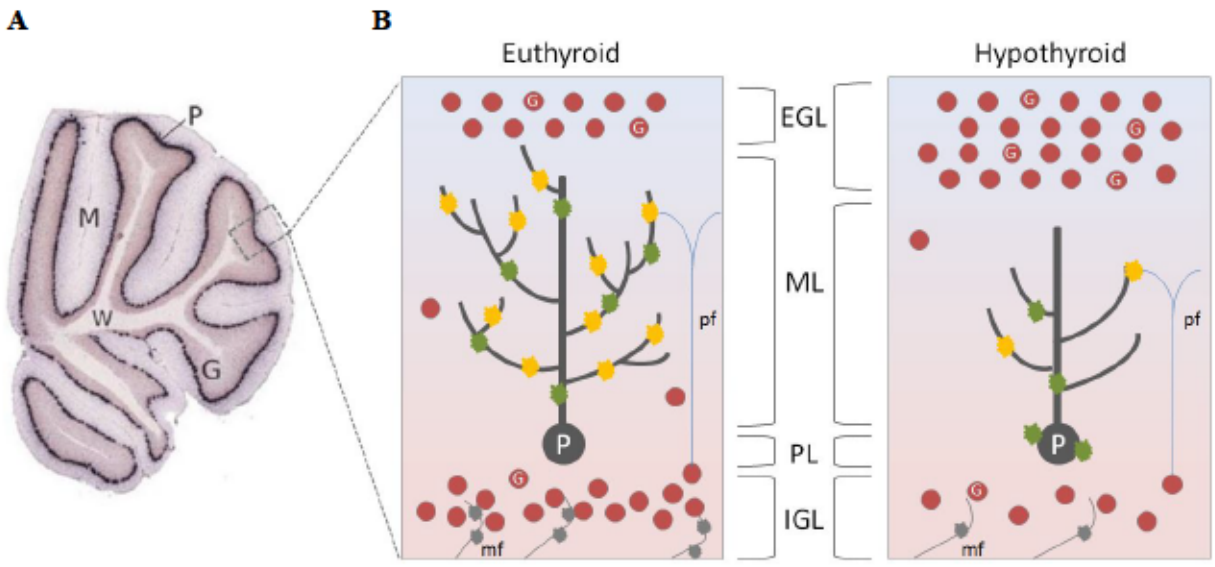


Fig.1.4 Schematic illustration of cerebellar cell types and layers under euthyroid and hypothyroid condition during the second postnatal week in rodents A) Cerebellum layers can be easily discriminated. Purkinje cell layer (P), molecular layer (M), granular layer (G) and white matter (W). **B)** Thyroid hormones impair strongly the dendritogenesis of the Purkinje cells (P) in the molecular layer (ML), the migration of the granular cells (red circles, also schown as `G`) from the external granular layer (EGL) to the inner granular layer (IGL) and the synaptogenesis with parallel fibers (pf, yellow) mossy fibers (mf, grey) and climbing fibers (green) (modified from Kirsch et al., 2012; Koibuchi and Chin, 2000).

In PC, intrinsic TRα1 signaling involves at least two modes of action. Binding of T3 to TRα1 can promote dendritic arborization, most likely by inducing the expression of genes involved in this process whereas unliganded TRα1 strongly blocks PC dendritogenesis. Interestingly, absence of TRα1 has very little effects on PC development. *In vitro* as well as *in vivo* studies of TRα1 ko mice revealed that GC and PC lacking TRα1 do not display signs of misdevelopment (Heuer and Mason, 2003; Morte et al., 2004). These results suggested that the aporeceptor activity of an unliganded TRα1 exerts the most devastating action and is responsible for the developmental impairments seen under hypothyroid conditions (Flamant et al., 2002). This is best illustrated in hypothyroid mouse mutants (so-called Pax8 mice) that show a rather normal cerebellar development if TRα is simultaneously inactivated (Flamant et al., 2002.)

Further, it was described that T3 promotes dendrite formation through induction of peroxisome proliferator-activated receptor gamma (PPARγ) co-activator1α (PGC-1α), a master regulator of mitochondrial biogenesis. PGC-1α expression is upregulated during dendritic outgrowth in

normal mice, while it is significantly retarded in hypothyroid mice or in cultures depleted of T3 (Hatsukano et al., 2017).

Another alteration in response to hypothyroidism is an impaired neurogenesis and neuronal differentiation due to an arrested neural stem cell cycling in the subventricular zone (SVZ) adjacent to the lateral ventricles, which generates olfactory bulb interneurons, and in the subgranular zone (SVZ), which gives rise to granular neurons in the hippocampal dentate gyrus (Eriksson et al., 1998; Alvarez-Buylla and Garcia-Verdugo, 2002). This leads to reduced neuronal cell number in the cortical layer and reduced cortical thickness (Mohan et al., 2012; Ming and Song, 2011). Moreover, hypothyroidism causes a delay and arrest of astrocyte differentiation as well as a decreased number of pyramidal cells in the cerebral cortex (Manzano et al., 2007). In addition, TH has been shown to play a fundamental role in the development of inhibitory circuits in the early postnatal rodent cerebral cortex and hippocampus. Especially, a subtype of GABAergic interneurons that expresses the calcium-binding protein parvalbumin (PV) exhibits a high sensitivity towards alterations in TH levels. PV-expressing (PV+) interneurons are basket and chandelier cells that form synapses with the soma or initial axonal segment of principal cells of hippocampus and neocortex. Activation of these local circuit neurons effectively limits the action potential firing by pyramidal cells of the cortex and hippocampus as well as GC of the dentate gyrus (Celio, 1986; Katsumaru et al., 1988). Hypothyroidism is associated with a delayed increase in GABA receptor density and a decreased number of PV+ cells and inhibitory function in the cerebral cortex and hippocampus (Gilbert et al., 2007; Wallis et al., 2008). Moreover, precursors of GABAergic interneurons show decreased proliferation and delayed differentiation under hypothyroid conditions (Nicholson and Altman, 1972). In rats, neonatal hypothyroidism induced a reduction in the activity of glutamic acid decarboxylase (GAD) in the developing cortex. This enzyme is responsible for the production of GABA in the CNS (Virgili et al., 1991).

Promoting myelination in the brain represents another important function of TH. Prolonged neonatal hypothyroidism in rodents causes a delayed and poor deposition of myelin in white matter brain areas with a decreased number of myelinated axons as TH signaling controls the proliferation, migration and maturation of myelin-forming oligodendrocytes (OL) (Fig.1.5). Thus, TH deficiency results in a reduced neuronal connectivity, leading to motor, sensory and cognitive deficits (Thompson et al., 2000; Calzà et al., 2018). TH treatment blocks the proliferation of OPC and induces their differentiation into mature OL. Further, TH signaling controls the synthesis of OL survival factors, such as neurotrophin-3 or insulin-like growth

factor-1 (Anderson et al., 2003). TH also enhances morphological and functional maturation of postmitotic OL and stimulates the expression of different myelin genes, such as myelin basic protein (mbp), proteolipid protein (plp), myelin-associated glycoprotein (mag) (Rodriguez-Pena et al., 1993; Barradas et al., 2001). The reduction of these myelin-proteins in hypothyroid brain likely plays a significant role in the observed effects of hypothyroidism on myelination (Thompson and Potter, 2000).

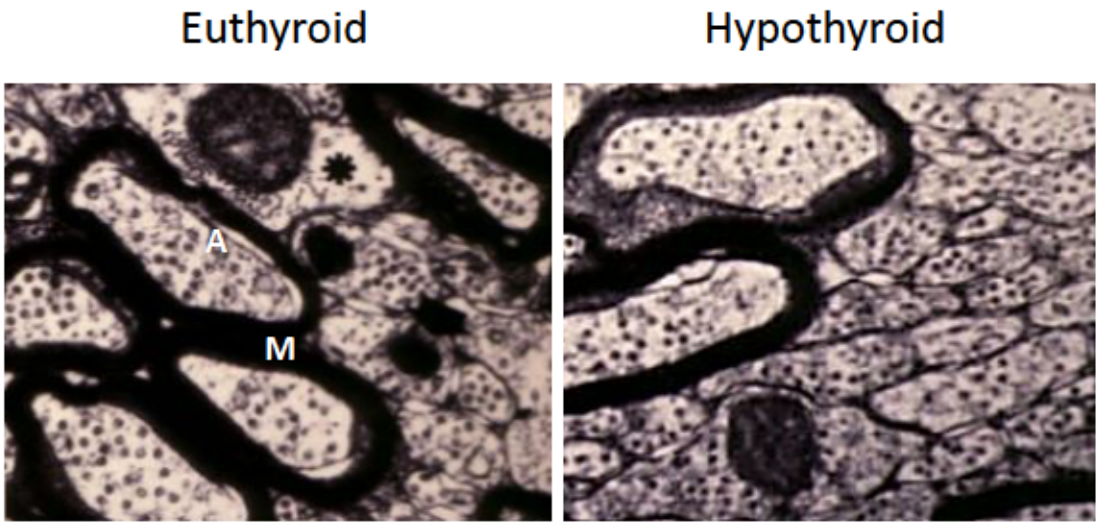


Fig.1.5 Myelination in the anterior commissure of the brain in euthyroid and hypothyroid rats. Hypothyroidism was produced during neonatal period and rats were analyzed at 6 months of age. The electro microscopy analysis shows a reduced number of myelinated (M) axons (A) in the hypothyroid rats (adapted from Berbel et al., 1994).

1.2 Thyroid hormone transport

Since metabolization of TH by deiodinases, the binding of T3 to its nuclear receptors and the regulation of gene expression are intracellular processes, TH have to cross the cell membranes. A vast number of studies suggest that TH require the presence of transporter proteins in the cell membranes that facilitate their cellular uptake and efflux. Until the 1970s, it was thought that the passage of TH occur via passive diffusion due to fact that TH with its lipophilic ring-structures could easily cross the lipid-rich bilayer of the cell membrane. However, TH are charged amino acid derivatives and therefore depend on specialized transport proteins to cross any lipid bilayer membrane (Hennemann et al., 2001).

TH entering the CNS from the cerebral circulation must first cross either the blood-brain barrier (BBB) or the blood-cerebrospinal fluid barrier (BCSFB) (Fig.1.6). In adult rodents, the BBB has been suggested to be the primary route for TH to enter the brain, while in the rat fetuses,

there is evidence that the BCSFB is the main route of TH transfer from blood to brain (Dratman et al., 1991; Johansson et al., 2008).

The BBB structure comprises endothelial cells, which are connected by tight junctions and surrounded by the end-feet of astrocytes and pericytes in the brain microvasculature (Aird, 2007; Shepro and Morel, 1993; Abbott et al., 2006). This barrier system, that starts to form at approximately 9.5 days post-conception in mice, controls and regulates the passage of molecules and ions and protects the brain from chemicals and pathogens (Pardridge, 1979; Obermeier et al., 2013; Rautio et al., 2008). The BCSFB is situated in the choroid plexus within each brain ventricle. In contrast to other cerebral blood vessels, the endothelial cells forming choroid plexus blood vessels are fenestrated and do not form a barrier. Here, the barrier-forming cells are the epithelial cells, which have tight junctions at their apical CSF side. Choroid plexus cells have microvilli on their apical side, increasing their exchange surface to the internal CSF (Brightman and Reese, 1969).

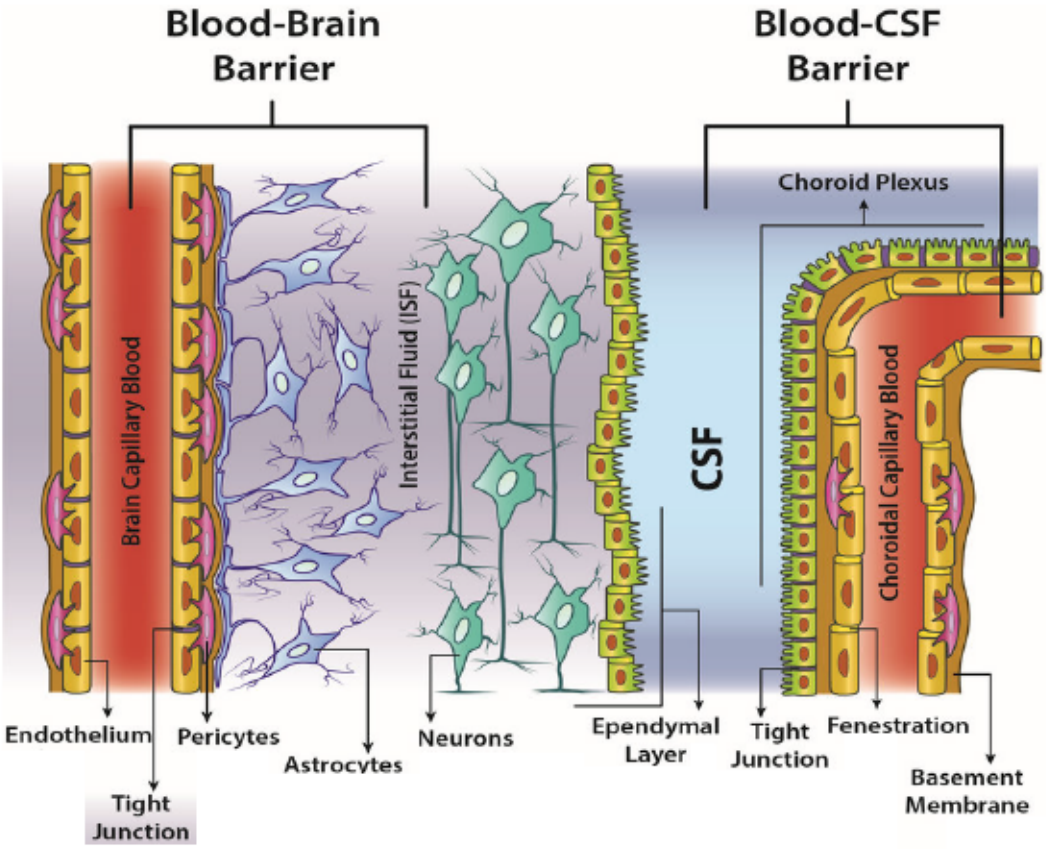


Fig.1.6 Schematic representation of the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB). The BBB is formed by brain endothelial cells in capillaries that are connected by tight junctions and are surrounded by pericytes and astrocytes at the basolateral side of the endothelial cells. The BCSFB is found in the choroid plexus of each ventricle of the brain and is built by fenestrated endothelial cells and a monolayer of tight-junctioned epithelial cells. This particular epithelium is in direct continuity with the ependymal layer lining the ventricle (adapted from D´Agata et al., 2017).

Thus, in order to access the CNS either via BBB or BCSFB, TH have to cross several cells and cell membranes.

Until now, several classes of transmembrane iodothyronine transporters from different gene families are known. One of the first identified TH transporters was the bile acid transporter sodium-taurocholate co-transporting polypeptide (NTCP/SLC10A1) (Friesema et al., 1999). Further, members of the large neutral amino acid transporter family, such as LAT1 and LAT2 have been shown to be responsible for the TH transport as well as several members of the organic anion transporting polypeptide (OATP) superfamily (Friesema et al., 2001; Hagenbuch, 2007). In addition, members of the monocarboxylate transporter (MCT) family, namely MCT8 and MCT10 have been shown to contribute to the TH transport (Friesema et al., 2003; Friesema et al., 2008). All this transporter can mediate the cellular influx or efflux of TH with different kinetics and substrate preferences (Bernal et al., 2015; Heuer and Visser, 2009; Heuer and Visser, 2013). Most of them, with the notable exception of MCT8, show a broad substrate specificity (Friesema et al., 2003).

1.2.1 Monocarboxylate Transporters

Monocarboxylate transporters (MCT) are a family that contains 14 identified members to date. They are present in various tissues and different species and the majority of them transport monocarboxylates such as pyruvate, lactate, ketone bodies and aromatic amino acids (Pierre and Pellerin, 2005; Bernal et al., 2015; Krassas et al., 2007).

The monocarboxylate transporter 8 (MCT8) is an energy-independent and bidirectional-active transporter that in contrast to other family members only accepts iodothyronine derivates as substrates. It is encoded by the SLC16A2 (solute carrier family 16, member 2) gene (OMIM 300095), localized on the X chromosome (Xq13.2 in human) and consists of six exons and five introns (Lafreniére et al., 1994; Friesema et al., 2003; Friesema et al., 2006). The protein contains twelve transmembrane domains, and both the N- and the C- terminus are located inside the cell (Jansen et al., 2005; Halestrap and Meredith, 2004). Human, rat and mouse MCT8/Mct8 share high sequence homology with only four amino acids changes and the insertion of a 20-amino acid repeat in the mouse sequence which is absent in the rat and human sequences (Friesema et al., 2003). Two different protein isoforms of 539 and 613 amino acids in humans are translated due to two different translational initiation sites. The shorter MCT8 protein (~60 kDa) functions as a TH transporter in human and rodents. The longer one (~70 kDa) is exclusively expressed in primates and its role is not yet understood (Jansen et al., 2005; Friesema et al., 2006). A first functional characterization of rat Mct8 (rMct8) in Xenopus laevis

oocytes revealed that the uptake of iodothyronines T4, T3, rT3 and T2 was strongly stimulated in the presence of Mct8 (Friesema et al., 2003). Consecutively, TH transport by Mct8 was also shown for zebrafish and chicken (Zada et al., 2014; de Vrieze et al., 2014; van Herck et al., 2015; Delbaere et al., 2017).

1.2.1.1 Monocarboxylate transporter 8

MCT8 expression has been detected in different species and numerous tissues. In rodents, Mct8 has been found in liver, kidney, thyroid gland, placenta and skeletal muscle (Friesema et al., 2003; Becker et al., 2010; Trajkovic et al., 2007; Trajkovic-Arsic et al., 2010; Müller et al., 2014; Vasilopoulou et al., 2013; Mayerl et al., 2018). Immunhistochemical studies and *in situ* hybridization experiments in the mouse brain, showed pronounced Mct8 in different neuronal populations of the cerebral and cerebellar cortex, hippocampus, striatum and hypothalamus, with higher expression during early postnatal stages. Further, Mct8 mRNA was detected in OL and astrocytes (Braun et al., 2011; Lee et al., 2017). Mct8 was also strongly expressed in capillary endothelial cells, choroid plexus structures as well as in tanycytes, which are spezialized ependymal cells lining the third ventricle (Wirth et al., 2009; Heuer et al., 2005; Ceballos et al., 2009; Roberts et al., 2008). This is in line with functional studies suggesting that Mct8 is important for the T3 transport across the BBB and BCSFB (Fig.1.7) (Trajkovic et al., 2007; Ceballos et al., 2009).

In humans, MCT8 mRNA levels are highest in liver and adrenal gland and slightly lower in a variety of other tissues including brain, kidney, placenta and thyroid (Roberts et al., 2008; Nishimura and Naito, 2008; Price et al., 1998, Friesema et al., 2012). Expression of MCT8 was also detected in neurons and astrocytes as well as in tanycytes (Alkemade et al., 2005; Kallo et al., 2012). Further, detailed analysis of MCT8 expression in human brain tissues from gestational week (GW) 14 to GW38 showed in all stages strong immunoreactivity within vascular structures in all brain regions. Moreover, MCT8 was present in the choroid plexus epithelial cells and fenestrated capillaries throughout development as well as in the adult CNS, whereas neuronal MCT8 expression appeared to be weak in the adult brain (López-Espindola et al., 2019; Robberts et al., 2008; Wirth et al., 2009).

The physiological importance of MCT8 as a TH transporter was recognized when mutations in the respective gene were identified in patients as the molecular cause for a severe X-linked intellectual disability (Friesema et al., 2004; Dumitrescu et al., 2004). The clinical phenotype of the affected males was reminiscent to the Allan-Herndon-Dudley Syndrome (AHDS),

originally described in 1944 by Allan, Herndon and Dudley (Allan et al., 1944). To date, at least 320 affected individuals in 132 families have been reported (OrphaNet, Allan-Herndon-Dudley Syndrome, OMIM 300523). Thereby, over 100 mutations of the MCT8 gene, including truncations, deletions, missense and nonsense mutations have been related to the AHDS (Groeneweg et al., 2017; Braun and Schweizer, 2018). This severe form of psychomotor retardation affects children from an early age (Schwartz et al., 2005; Schwartz and Stevenson, 2007). They suffer from a combination of neurological symptoms that include profound proximal hypotonia with poor head control, dystonic movement and spastic paraplegia as well as the lack of speech and poor motor communication skills with the disability to sit or stand independently (Schwartz et al., 2005; Visser et al., 2009; Novara et al., 2017). All affected males display intelligence quotient values mostly below 40 and despite a normal head circumference at birth, some patients present microcephaly with advancing age (Schwartz et al., 2005; Visser et al., 2011). Moreover, delayed myelination has been described by magnetic resonance imaging (MRI) in almost all affected children below five years of age and has been considered as a sign of central hypothyroidism (Tonduti et al., 2013; Sijens et al., 2008). Further, studies have revealed delayed cortical and cerebellar development, impaired axonal maturation as well as loss of PV expression (Espindola et al., 2014).

In addition to the severe global developmental delay, all patients with MCT8 mutations exhibit abnormal serum TH parameters, in particular highly elevated serum T3 levels and decreased T4, free T4 and rT3 concentrations (Dumitrescu et al., 2004; Schwartz et al., 2005; Friesema et al., 2004). Despite the highly elevated serum T3 levels, serum TSH is slightly increased rather than suppressed, suggesting that the pituitary does not sense the high serum T3 levels (Friesema et al., 2006).

Different hallmarks of a peripheral thyrotoxic state have been described, such as reduced body weight and length, low body fat content and tachycardia (Zung et al., 2011; Friesema et al., 2004; Groeneweg et al., 2019). Further, clinical studies disclosed an increased level of sex-hormone-binding globulin (SHBG), indicating a hyperthyroid situation in the liver, as this protein is strongly stimulated by T3 (Pugeat et al., 1996; Friesema et al., 2006). Moreover, muscle wasting is frequently observed in AHDS patients that might also reflect peripheral hyperthyroidism of skeletal muscle. In this regard, Herzovich et al. observed high levels of both lactic acid and ammonium in affected patients and postulated its indication for muscle wasting and body weight loss (Herzovich et al., 2007).

So far, the underlying mechanisms leading to this combination of abnormal TH levels and severe neurological deficits are not fully understood and information about the cellular damage in the brain is still limited.

1.2.1.2 Monocarboxylate transporter 10

The monocarboxylate transporter 10 (MCT10), also known as T-type amino acid transporter (TAT1), is encoded by SLC16A10 gene. With an amino acid sequence identity of up to 49%, it represents a close relative to MCT8. Studies with mammalian cells transfected with cDNA coding for human (h) MCT10 showed its ability to facilitate the transport of TH with a preference for T3, but in contrast to hMCT8 accepts additionally aromatic amino acids such as phenylalanine, tyrosine and tryptophan (Friesema et al., 2008, Friesema et al., 2006). Under identical conditions, hMCT10 is slightly more efficient than hMCT8 at transporting T3 (Friesema et al., 2008).

Expression studies in mice revealed the localization of Mct10 protein at the basolateral membrane of small intestine epithelial cells, of renal tubule cells and in hepatocytes (Ramadan et al., 2006; Ramadan et al., 2007). In the developing mouse brain only weak mRNA signal intensities for Mct10 were observed, while in the adult mouse brain Mct10 was enriched in white matter and dentate gyrus of the hippocampus (Müller and Heuer, 2014). In the human fetal brain, weak expression of MCT10 was detected at 10 weeks gestation, whereas analysis of the adult brain exhibited strong expression in the hypothalamus (Chan et al., 2011; Alkemade et al., 2011). So far, no patients with MCT10 mutations have been reported. Mct10 deficient mice showed increased aromatic amino acids concentration in kidney and plasma but no alterations in TH homeostasis (Mariotta et al., 2012).

1.2.2 Organic anion transporting polypeptides

Another well-characterized transport system is the sodium-independent organic anion-transporting polypeptide (OATP) family that is classified within the solute carrier family SLCO. OATP are polyspecific for amphipathic organic compounds such as steroid hormones, bile salts, xenobiotics and anionic oligopeptides. Further, they are energy-independent and act in a bidirectional manner (Visser et al., 2011). Up to date, more than 40 different OATP have been found in humans, rats and mice but just one member of the OATP family, the OATP1C1 (SLCO1C1) has been characterized as a TH transporter *in vivo,* so far. This transporter, who´s genetic information is encoded on chromosome 6 in mice or chromosome 12 in humans, consisting 15 exons, has a molecular protein mass of ~75 kDa and shows highest specificity

and affinity for iodothyronines T4 and rT3 (Pizzagalli et al., 2002). In humans, OATP1C1 is expressed in the Leydig cells of testes and the ciliary body of the eye (Pizzagalli et al., 2002; Gao et al., 2005). Its mRNA is widely abundant in human brain with highest expression in the cerebral cortex, amygdala and hippocampus, but not in the cerebellum (Pizzagalli et al., 2002). In particular, OATP1C1 protein is present in epithelial cells of the choroid plexus and in tanycytes. Only weak expression is found in the capillary vessels of the BBB, although OATP1C1 immunoreactivity was detected in astrocytes surrounding these vessels (López-Espindola et al., 2019). In rodents, Oatp1c1 is mainly expressed in brain and in some other tissues, such as placenta and the eye (Gao et al., 2005; Akanuma et al., 2013). In the adult rodent brain, Oatp1c1 is localized in subpopulations of astrocytes and tanycytes as well as in epithelial cells in the choroid plexus, where it is co-expressed with Mct8 (Tohyama et al., 2004; Heuer et al., 2005; Heuer and Visser, 2009). Contrarily to the human situation, Oatp1c1 is also present at the membrane in vascular endothelial cells of the BBB in the rodent CNS (Mayerl et al. 2012; Sugiyama et al., 2003; Roberts et al., 2008). Therefore, it has been suggested to be important for the passage of T4 from the circulating blood system to the brain (Fig.1.7). Moreover, Oatp1c1 is expressed in astrocytic subpopulations, predominantly in distinct brain areas, such as the hippocampus (Gao et al., 2005).

The relevance of OATP1C1 for physiology is not fully understood. Genetic variations in the human OATP1C1 gene were not associated with changes in the serum TH profiles (van der Deure et al., 2008a). However, polymorphisms in OATP1C1 were associated with subtle changes in clinical endpoints, such as depression and fatigue in T4-substituted hypothyroid patients (van der Deure et al., 2008b). Additionally, one patient with a homozygous mutation in OATP1C1 has been recently identified, showing features of brain hypothyroidism combined with neurodegeneration (Strømme et al., 2018; Groeneweg et al., 2019).

1.2.3 L-type amino acid transporters

The L-type amino acid transporters (LAT) comprise a heterogenous family of proteins that mediate as antiporters the exchange of neutral amino acids, such as leucine, phenylalanine and tyrosine in a sodium-independent way (Palacin, 1994; Mastroberardino et al., 1998). The Large neutral amino acid transporter small subunit 1 (LAT1, encoded by SLC7A5) and the Large neutral amino acid transporter small subunit 2 (LAT2, encoded by SLC7A8) are two members of this family. They are heterodimeric and consist of a common heavy chain (4F2hc) and a homologous light chain. The heavy chain is a glycolysated protein with a single transmembrane domain, whereas the light chain is not glycolysated and has 12 transmembrane domains. They

are linked through a disulfide bond (Palacin, 1994; Verrey et al., 1999). LAT were shown to accept iodothyronines as substrates. In particular, LAT1 is efficient in transporting T3, whereas LAT2 has been shown to be effective in transporting the TH metabolite T2 (Pinho et al., 2003; Bernal et al., 2015; Mastroberardino et al., 1998). Both transporters are widely expressed in the developing mouse brain, in particular in neurons, astrocytes, oligodendrocyte precursor cells and microglia and very abundant in endothelial cells of the microvasculature (Bernal et al., 2015; Verrey et al., 1999; Mastroberardino et al., 1998). In the human brain LAT1 was predominantly described in the cerebral cortex and the BBB (Nakada et al., 2014), while LAT2 was mainly expressed in the cerebral cortex and in the hippocampus (Pinho et al., 2004; Kobayashi, 2007). Besides the brain, LAT have been identified in many other tissues such as intestine, kidney, placenta, testis and ovary in both, humans and rodents (Pinho et al., 2004).
Alterations in the LAT expression have been associated with different diseases. The overexpression of LAT1 and LAT2 is described in human cancer as a prognostic factor of metastasis as well as a high-risk condition for insulin resistance at birth and for developing type-2 diabetes (Wang and Holst, 2015; Cormerais et al., 2016; Kobayashi et al., 2018). Contrarily, LAT1 expression is reduced in Intra-Uterine Growth Restriction (IUGR) leading to a high risk of cardiovascular and metabolic diseases in childhood (Rosario et al., 2011; Scalise et al., 2018). Regarding the BBB, decreased expression of LAT1 is linked to the onset of Parkinson´ s disease as well as to Autism Spectrum Disorder (ASD), highlighting its crucial role in several human pathologies (Scalise et al., 2018).

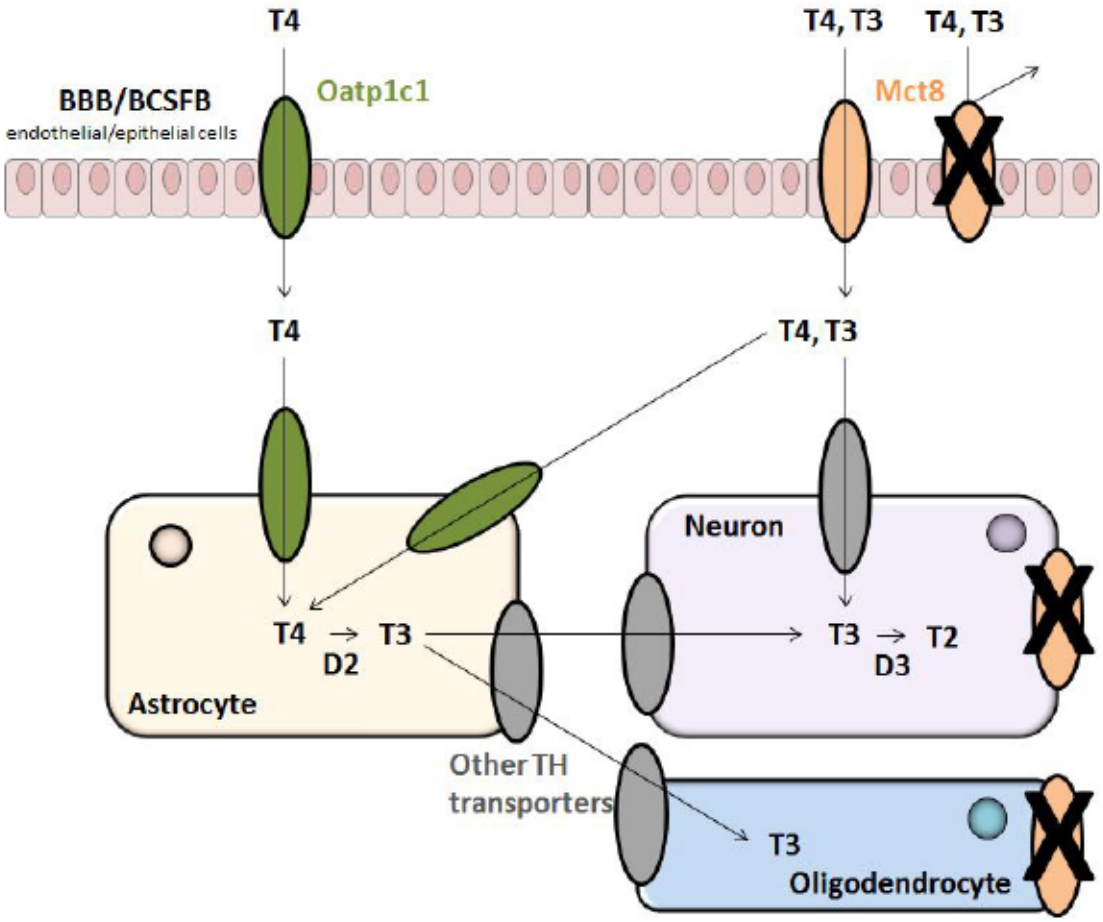


Fig.1.7 TH transport controls TH availability and action in the mouse brain. Several transporters are necessary to facilitate the uptake of T3 and T4 from blood to brain across the BBB and BCSFB. In the absence of Mct8, T3 transport is compromised, but T4 is still transported via Oatp1c1 that is also present in the astrocyte endfeet and in close contact with the endothelial cells. T4 in the astrocytes acts as a substrate for D2, which provides the brain with enough T3 to compensate for the lack of T3 transport. Metabolism of T3 takes place in neurons where T3 is deactivated into T2 by D3. Efflux of T3 from astrocytes and influx into neurons and oligodendrocytes is mediated by other TH transporters such as LAT, Mct10 or members of the Oatp family.

1.2.4 Generation of Mct8/Oatp1c1 dko mice

To study the pathological mechanisms of AHDS in patients with MCT8 mutations, global Mct8 ko mice have been created as an experimental model of the disease. Characterization of these mice revealed that Mct8 ko mice faithfully replicate the peripheral phenotype of patients with MCT8 deficiency and shed light on several pathogenic mechanisms. In particular, it was shown that Mct8 ko animals have markedly decreased T4 and rT3 levels and highly elevated serum T3 concentrations leading to a thyrotoxic situation in many peripheral tissues, such as liver, kidney, skeletal muscle and bone (Dumitrescu et al., 2006; Trajkovic et al., 2007; Mayerl et al., 2018; Leitch et al., 2017). These data demonstrate that T3 can enter different target cells independent of Mct8. Further, the thyroid gland itself shows enlarged follicles and increased

thyroglobulin- bound and free TH in the absence of Mct8 (Trajkovic et al. 2010; Di Cosmo et al., 2010; Wirth et al., 2011). TSH stimulation tests in Mct8 ko mice demonstrated a reduced thyroidal secretion of T4, indicating that Mct8 acts as a T4 efflux system in the murine thyroid gland whereas T3 is primarly released by another transport system (Trajkovic et al., 2010; Di Cosmo et al., 2010; Müller et al., 2014). In contrast to peripheral tissues, the uptake of radiolabeled T3 into the murine brain was strongly diminished in Mct8 ko mice (Trajkovic et al., 2007). Regarding the localization of Mct8 in the choroid plexus and brain endothelial cells these data suggest a dominant role for Mct8 in the transport of T3 across the BBB (Wirth et al., 2009; Dumitrescu et al., 2006; Trajkovic et al., 2007). The uptake of labeled T4 was only slightly affected, due to a compensatory increase in D2 activity together with a decrease in D3 activity in the CNS of the mutant mice (Trajkovic et al., 2007). The central TH content in Mct8 ko mice is reduced to 60% of control animals and the expression of directly TH-regulated genes is only mildly affected upon Mct8 deficiency. Overall, these animal studies revealed that Mct8 is not the only TH transport system in mice since Mct8 ko mice appear normal without any visible growth retardation, altered behavior or neurological deficits. Mct8 ko mice also do not show abnormalities in PC development or in the differentiation of inhibitory neurons in the cerebral cortex indicating that these cells are supplied with TH even in the absence of Mct8 (Trajkovic et al., 2007; Heuer et al., 2005; Wirth et al., 2009).

Obviously, another TH transporter, putatively Oatp1c1, can partially compensate for the absence of Mct8 in the mouse CNS whereas in the human CNS such a compensatory TH transport system is missing. Intriguingly, Oatp1c1 is strongly expressed at the murine BBB and choroid plexus structures but absent in human BBB endothelial cells (Tohyama et al., 2004; Müller and Heuer, 2014; Roberts et al., 2008). Indeed, the generation of the Mct8/Oatp1c1 double knockout (dko) mice confirmed the physiological significance of both TH transporters for proper TH homeostasis in the murine brain. In these animals, TH uptake into the CNS was strongly diminished to around 10% of Wt levels (Mayerl et al., 2014). Accordingly, M/O dko brains were found in a TH-deficient state, which resulted in clear defects in brain development and marked neurological abnormalities. Unlike the single mutant animals, M/O dko mice exhibit pronounced behavioral alterations including an ataxic gait and reduced locomotor performance. In the brain, they showed a reduced number of PV- expressing cells in the somatosensory cortex, a delayed cerebellar PC dendritogenesis as well as a reduced myelination (Mayerl et al., 2014). Taken together, the M/O dko mouse is the first animal model exhibiting a pronounced brain phenotype that in addition fully replicates the thyroidal serum abnormalities characteristic for patients with AHDS and may, therefore, be considered as a suitable animal

model for studying human MCT8 deficiency and testing therapeutic interventions (Müller and Heuer, 2014).

1.3 Therapeutic options

Since the genetic cause of AHDS has been elucidated, many patients with MCT8 mutations have been identified worldwide. With this increasing prevalence, the need for therapeutic options rises, but to date, no curative therapy is available for MCT8 deficiency. Possible treatment options for patients suffering from this disease are rather limited and the development of an appropriate treatment strategy is challenging, because patients present cerebral hypothyroidism together with peripheral hyperthyroidism (Kersseboom et al., 2014; Visser et al., 2011). Therefore, therapeutic strategies should improve the neurological defects derived from TH deficiency in the brain and alleviate the peripheral thyrotoxicosis (Bárez-López et al., 2018).

Many AHDS patients have been treated with levothyroxine (LT4), due to their low serum T4 and high normal to slightly elevated TSH levels that were interpreted as mild hypothyroidism. However, LT4 administration did not result in any clinical improvement and even leaded to a further increase in serum T3 levels aggravating the thyrotoxic state of peripheral tissues in these patients (Zung et al., 2011; Kersseboom et al., 2011; Biebermann et al., 2005).

In contrast, the combination of propylthiouracil (PTU) together with LT4 replacement resulted in a normalization of TH serum levels. Thereby PTU was chosen to block endogenous TH production and inhibit D1 activity, while LT4 was given to increase TH levels. This therapy leads to an improvement in the peripheral symptoms of thyrotoxicosis, such as an increased heart rate and body weight loss. Unfortunately, neurological improvements were not observed due to the impaired TH uptake into the CNS and long-term administration of PTU harbours a risk of severe side-effects, such as agranulocytosis and liver failure (Wémeau et al., 2008; Visser et al., 2013).

The absence of beneficial effects of LT4 treatment on the neurocognitive features prompted studies to alternative therapeutic approaches. The current efforts to develop treatment strategies are focusing on overcoming the limitations at the BBB, since it represents a main restriction for TH entry into the target neural cells and is a unique challenge for CNS drug development as it can significantly restrict therapeutic exposure from systemic circulation. Therefore, another possibility to counteract the neurological damage is the treatment of patients with bioactive TH

analogs that mimic the action of TH but do not rely on MCT8 for their distribution to the CNS (Groeneweg et al., 2017a,b; Bárez-López et al., 2018; Meinig et al., 2019).

1.3.1 Ditpa

TH are known to have a cholesterol-lowering effect in hypothyroid individuals, but their pharmacological use for this purpose is limited due to their actions on other organs, including heart, bone and brain where they can induce side effects of excessive TH action (Tancevski et al., 2009). In the 1960s large numbers of TH analogs, including the 3,5-diiodothyroprionic acid (Ditpa), were synthesized for using their cholesterol-lowering property in euthyroid individuals without affecting the heart (Blank et al., 1963). In addition, Ditpa has been shown to increase cardiac performance and to improve left ventricular function in post infarction experimental models of heart failure before it has been the first TH analog studied in the context of MCT8 deficiency (Pennock et al., 1993; Di Cosmo et al., 2009).

Ditpa is a compound with low metabolic activity and binds with approximately equal affinities to all TR isoforms but with a 300-fold lower affinity than T3 (Pennock et al., 1992; Barker et al., 1951). Administration of Ditpa to Mct8-deficient and control mice resulted in a similar tissue availability of Ditpa in liver and brain where it showed promising effects on normalizing expression levels of TH-regulated genes. These data suggested that Ditpa enters these tissues independent on Mct8 and represents a suitable T3 substitute in the absence of Mct8 (Di Cosmo et al., 2009; Tonduti et al., 2013). Furthermore, Ferrara and colleagues demonstrated that Ditpa at a dose of 3 µg/g bw can cross the placenta of hypothyroid pregnant mice and exert thyromimetic effects in the fetal cerebral cortex as indicated by gene expression analysis of TH-responsive genes (Ferrara et al., 2014). Application of Ditpa for 26-40 months to four children (8.5-25 months old) with MCT8 deficiency resulted in a normalization of serum T3 and TSH and increased the serum T4 and rT3 to low normal or slightly below normal levels without causing any adverse effects (Verge et al., 2012). Moreover, Ditpa treatment changed several parameters of TH actions, as it caused a decline in serum SHBG concentrations, reduced the heart rate stimulated weight gain (Verge et al., 2012). Despite these beneficial effects, no improvement of mental and motor function was achieved (Groeneweg et al., 2017a; Goldman et al., 2009; Verge et al., 2012).

1.3.2 Triac

Another promising T3 analog for the treatment of AHDS patients is triiodothyroacetic acid (Triac, TA3) (Groeneweg et al., 2017c). It represents a naturally occuring T3 metabolite and has been proven to be transported independent of Mct8 (Kersseboom et al., 2014). Triac binds *in vitro* to TR with a similar affinity as T3 to TRα1 and a 10-fold higher affinity than T3 to TRβ1 and TRβ2 (Messier and Langlois, 2000). Further, T3 and Triac seem to have similar metabolic pathways since they are both effectively metabolized by D3 (Kersseboom et al., 2014). However, little is known about its physiological role as well as factors that influence its serum and tissue distribution (Kersseboom et al., 2014).

Administration of Triac was already successfully used to suppress TSH in patients with TH cancer and for treating TH resistance due to TH receptor-β mutations (Müller-Gärtner et al., 1988; Radetti et al., 1997; Anzai et al., 2012).

Moreover, data from preclinical studies suggest that Triac restores abnormal neuronal development, in particular stimulating the dendritic arborization of cerebellar PC and normalizes hypomyelination in Mct8-deficient animal models if given in early postnatal life (Horn et al., 2013; Kersseboom et al., 2014).

Further, it ameliorates the development of cerebellum in the Pax8 ko mice that are born without a functional thyroid gland and therefore represent an animal model for congenital hypothyroidism (Mansouri et al., 1998; Horn et al., 2013). A major disadvantage for the therapeutic application of Triac is its short half-life in the circulation. In humans, Triac has a half-life of only approximately 6 to 8 hours compared to T3 with half-life of 23 hours (Menegay et al., 1989). In rats the half-life of Triac at 5.5 hours is even shorter (Liang et al., 1997).

Recently, Groeneweg and his colleagues started the first clinical trial based on these promising preclinical studies (Groeneweg et al., 2019). Thereby, they investigated the effectiveness and safety of oral Triac for peripheral thyrotoxicosis in 45 male pediatric and adult patients with MCT8 deficiency. Triac was administered with an initial dose of once-daily 350 µg and was then increased progressively in 350 µg increments to attain serum total T3 concentrations within the target range of 1.4-2.5 nmol/L. Patients were evaluated for study outcomes at baseline and 12 months after starting Triac treatment. Therefore, they were screened for clinical and biochemical signs of hyperthyroidism, recorded adverse events and adherence to therapy. It was shown that Triac treatment decreased the highly elevated serum T3, T4 and TSH concentrations at month 12, with beneficial effects on the thyrotoxic state of peripheral tissues. As a consequence, body weight increased, heart rate and systolic blood pressure decreased and hypertension were resolved in most of the patients receiving Triac treatment. In addition, serum

markers for tissue TH state, including SHBG concentration improved. Furthermore, explorative analysis showed an improvement in motor function in patients who started the Triac treatment before 4 years of age. The ability of Triac to improve the neurological impairments in very young infants is currently under investigation and will be the topic of a second Triac trial that is currently in preparation (ClinicalTrials.gov Identifier: NCT02060474) (Groeneweg et al., 2019).

1.3.3 Sobetirome

In order to further improve drug delivery and optimize thyromimetic action in the brain, also other TH analogs have been studied, including sobetirome. Sobetirome (also known as GC-1) is a synthetic derivative of TH and a potent T3 mimic. Its synthesis was reported 1998 by Chiellini and collegues (Chiellini et al., 1998). The principal structural changes presented by sobetirome, with respect to T3 were the three iodine residues that were replaced by the methyl and isopropyl groups, a methylene linkage replaced by the biaryl-ether linkage between the two phenol groups and the amino acid side chain at position 1 that was exchanged to an oxyacetic group (Fig.1.8) (Chiellini et al., 1998; Scanlan, 2010).

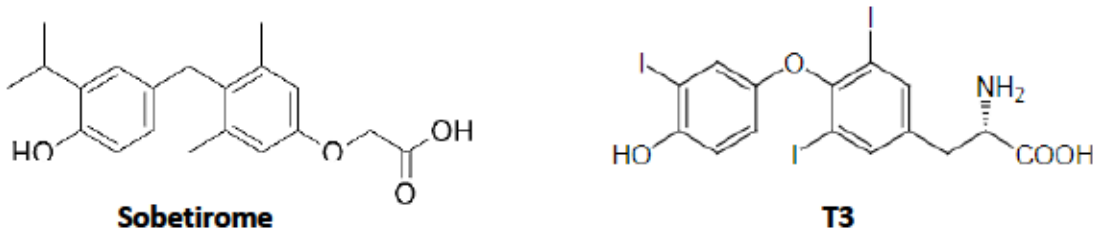


Fig.1.8 Chemical structures of sobetirome (GC-1; Sob1) and T3.

Like Triac, sobetirome binds *in vitro* to TR with a 10-fold subtype selectivity for TRβ over TRα. It has been suggested that the TRβ selectivity relates to the presence of the oxo-acetate at position 1. This oxo-acetate group forms enhanced polar interactions with conserved arginine residues in a hydrophilic part of the TRβ pocket (Baxter et al., 2001; Baxter et al., 2009; Bleicher et al., 2008).

Originally, sobetirome has been tested extensively in preclinical animal models, including rodents and primates and reached Phase Ib in human clinical trials for the treatment of high

lipid levels (Trost et al., 2000; Grover et al., 2004). In one of the first investigations, sobetirome was administered to hypothyroid mice and hypercholesteremic rats to compare its effects on cardiovascular action and lipid levels with that of equimolar doses of T3 (Trost et al., 2000). This study showed encouraging results, as serum cholesterol- and triglyceride concentrations were effectively reduced with sobetirome without any harmful effects on the heart rate or signs of thyrotoxicity. The potential mechanism underlying these responses was explained to be linked to differences in TRβ isoform distribution in specific organs (Trost et al., 2000). Previous data have shown that TRβ receptors are the predominant isoform in the liver, accounting for 80% of T3 receptor binding (Schwartz et al., 1992). Therefore, sobetirome as a TRβ-specific TH analog accumulates preferentially in the liver instead of in the heart, which allows its contribution to this marked lipid lowering effect without any effect on heart rate (Trost et al., 2000).

Further, it reduced levels of lipoprotein a, which is linked to atherogenesis, in primates (Grover et al., 2004). Studies of Lin and colleagues additionally, pointed to an independent lipid-lowering effect of sobetirome on the mechanism of the hepatic low-density lipoprotein receptor (LDLR) (Lin et al., 2012). This effect was attributed in reverse cholesterol transport mediated by increased expression of the high-density lipopreotein (HDL) receptor and present an evidence that this process is related to an increased conversion of cholesterol to bile acids and a subsequent increase in bile acid secretion, suggesting sobetirome as a promising cholesterol-lowering therapeutic approach for familial hypercholesterolemia (Lin et al., 2012).

Sobetirome has also been used as a possible treatment approach for nonalcoholic fatty liver disease (NAFLD) by preventing the development of hepatic steatosis and promoting a rapid regression of preexisting fat accumulation (Perra et al., 2008). Although the amelioration of fatty liver by this TH analog was evident in mice and although the treatment prevented hepatic steatosis in high-fat diet-fed rats, it caused hyperglycemia and insulin resistance, suggesting caution before implicating its beneficial effects in NAFDL (Martagón et al., 2015; Vatner et al., 2013).

Another study of constant and controlled administration of sobetirome was shown to reverse very high-fat diet-induced fat accumulation in the liver, induce weight loss, reduce fat mass and normalize glycemia as well (Filgueira et al., 2016; Columbano et al., 2017).

Based on its structure it has been proposed that sobetirome can cross the BBB by passive diffusion and distribute to the CNS at levels that are sufficient to upregulate T3 target genes

(Placzek et al., 2016). At least, a transport system that facilitates sobetirome passage across cell membranes has not been identified so far.

In the CNS, application of sobetirome has been considered for the treatment of X-linked adrenoleukodystrophy (X-ALD), a lipid storage disease of the brain and the adrenal gland. It has been shown that sobetirome treatment rapidly induces transcription of the membrane lipid transporter ABCD that in turn results in a reduced accumulation of very long chain fatty acids (VLCFA) in both periphery and CNS (Genin et al., 2009; Hartley et al., 2017). Furthermore, sobetirome has been shown to promote human and rodent oligodendrogenesis *in vitro* and to stimulate oligodendrogenesis during development in rodents, thereby increasing the production of myelin proteins *in vivo*. These beneficial effects support sobetirome as useful candidate for treating demyelinating disorders, such as multiple sclerosis (MS) (Baxi et al., 2014; Devereaux et al., 2018).

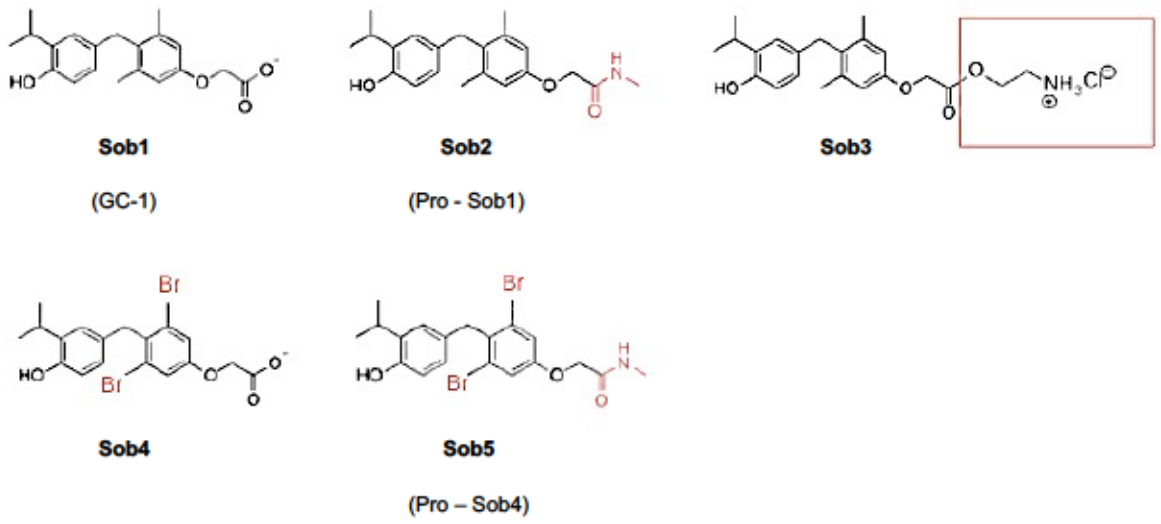


Fig.1.9 Chemical structures of the synthetic thyromimetics Sob1 – Sob5 (Chiellini et al, 1998; Placzek and Scanlan, 2015; Meinig et al., 2017; Ferrara et al., 2017; Devereaux et al., 2016).

1.3.4 Sobetirome modifications

Although sobetirome is a very promising drug candidate for CNS indications, its potency is lower than that of T3. A more potent analog would evoke the same effects at a lower dose or greater stimulation of the target at same dose (Devereaux et al., 2016).

Modification of the sobetirome structure (Fig.1.9) by replacing the 3,5-dimethyl groups with a bromine (Sob4 and Sob5) potentially exploits halogen bonding interactions with the TR ligand-

binding domain and increases significantly efficacy in binding affinity and selectivity *in vitro* and *in vivo* (Devereaux et al., 2016).

Most thyromimetics, including sobetirome, contain an inner ring, negatively charged carboxylate groups, which are crucial for high affinity binding to the TH receptor. A known liability for CNS drug distribution is their inherent lipophobic character and electrostatic repulsion at negatively charged tight junctions of BBB endothelial cells. While sobetirome does distribute to the CNS, more recently prodrugs of sobetirome have been developed that mask the carboxylate acid. Therefore, sobetirome prodrugs provide greater access to the CNS and limit peripheral exposure of the parent drug (Ferrara et al., 2017).

Ester and amide prodrugs of carboxylic acid containing drugs have been shown to increase CNS drug distributions following enzymatic hydrolysis in the CNS. Esters have generally an ideal hydrolysis rate that provides maximal CNS drug distribution (Rautio et al., 2008). After crossing the BBB, the prodrug esterases can cleave the prodrug ester liberating the active drug sobetirome in the CNS (Fig.1.10) (Placzek et al., 2016).

Amide prodrugs of sobetirome such as Sob2, leads to increased sobetirome concentrations across all brain regions, approximately 9-fold higher than the parent drug with decreased peripheral sobetirome exposure (Bárez-López et al., 2018). Until now, the uptake of sobetirome and its modified compounds into the CNS has not been elucidated yet.

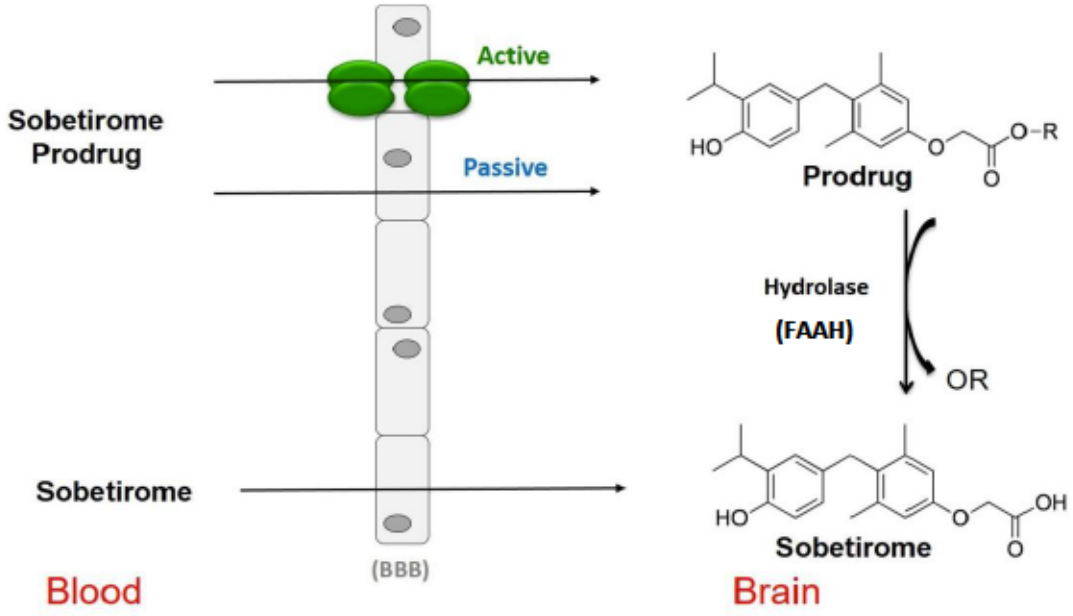


Fig.1.10 Prodrug strategy and cleavage for enhanced brain uptake of sobetirome. Sobetirome prodrugs cross the BBB and are cleaved by the non-specific FAAH, liberating the active drug sobetirome in the CNS (adapted from Placzek et al., 2016).

Recent studies of CNS distribution of sobetiramides have shown that Sob2 is hydrolyzed by Fatty Acid Amid Hydrolase (FAAH) (Meinig et al., 2019). FAAH is a membrane-bound serine hydrolase which generally catalyzes the hydrolysis of various fatty acid amids, including the endocannabinoid ananadamide (AEA), the hypnotic oleamide (OEA), the anti-inflammatory N-palmitoylethanolamine (PEA), and N-arachidonoyltaurine (Egertová et al., 2003; Boger et al., 2000). Its immunoreactivity could be detected throughout the brain, with the most intense expression in large, principal cells such as the pyramidal cells in the neocortex and the PC in the cerebellum (Tsou et al., 1998). It was shown that peripheral dosing of Sob2 generates large concentrations of sobetirome selectively in the brain compared to peripheral organs, with a considerable 60-fold increase to the brain/serum ratio compared to a peripheral dose of the parent drug sobetirome (Meinig et al., 2019). For these reasons sobetirome prodrugs are worth examining as therapeutic agents for CNS disorders, including AHDS that could benefit from TH action (Meinig et al., 2017).

1.4 Aim of the study

TH are essential for the proper development of multiple organs, notably the brain. To fulfill their function in the target cells TH have to cross a variety of membranes. Thereby, specialized transport proteins facilitate the uptake of TH into cells (Visser et al., 2008). Their importance gets especially clear in patients with inactivating mutations in the MCT8 gene that leads to a severe clinical phenotype of AHDS most likely due to massive reduction in CNS TH supply and a consequently disturbed differentiation and maturation of neural cells (Schwartz et al., 2005).

However, currently, there is no effective treatment available for AHDS, therefore there is an urgent need for the development of novel drugs (Shaji, 2018). A possible therapeutic approach for patients is the use of TH analogs that enter the brain independently of MCT8 and activate TH receptors in CNS target cells. Two TH analogs, Triac and Ditpa have already been tested in mouse models as well as in patients and both were successful in normalizing the peripheral thyrotoxic state. However, preliminary data indicate that both drugs have very little beneficial effects regarding the neurological symptoms of the patients (Verge et al., 2012; Horn et al., 2013; Groeneweg et al., 2017c). Thus, additional TH-like compounds should be considered that ideally exert strong thyromimetic effects in the CNS without causing peripheral side effect.

The aim of this study was to assess the capacity of novel Sob analogs in stimulating TH-dependent neuronal differentiation in comparison to T3. As as cellular primary system, cerebellar cultures of neonatal mice were used as cerebellar PC outgrowth as well the number of PV+ neurons are highly dependent on proper TH supply. Given that these Sob compounds exert even stronger TH-like effects in neurons they should be tested *in vivo* by taking advantage of Mct8/Oatp1c1 ko mice. To this end, these studies should unravel whether and to which extend novel TH analogs can be considered as a better treatment option than Ditpa and Triac for AHDS patients.

2 Materials and Methods

2.1 Animal breeding

In the present study, wild-type (Wt) C57/BL6 mice as well as mice with a combined germline deletion in the TH transporters Mct8 (Mct8 ko, MGI: 3710233) and Oatp1c1 (Oatp1c1 ko, MGI: 5308451) were used. Mct8 ko mice were originally obtained from Deltagen (San Mateo, CA) (Trajkovic et al., 2007). Conditional Oatp1c1 fl/+ mice on C57BL/6 background were generated at TaconicArtemis (Cologne, Germany). In order to inactivate the Oatp1c1 gene ubiquitously, Oatp1c1 fl/fl mice were crossed with C57BL/6 mice carrying Cre recombinase under the pCX promoter as described elsewhere (Mayerl et al., 2012). In addition, heterozygous Mct8 mutant female mice were mated with heterozygous Oatp1c1 ko/fl mice to generate Mct8 ko/Oatp1c1 ko (Mct8/Oatp1c1 dko, or M/O dko) offspring, as well as their respective control male mice exhibiting the genotype Mct8 +/y/Oatp1c1 fl/fl (Mayerl et al., 2014).

Primary cell culture experiments were performed with newborn Wt animals. All animals were provided with standard laboratory chow and tap water *ad libitum* and were kept in IVC cages under pathogen-free conditions in accordance with local regulations (LANUV NRW, Germany; AZ: 84-02.04.2015 A331) at constant temperature (22 °C) and alternating light cycle (12 h light, 12 h dark).

2.2 Genotyping

Ear biopsy or tail clip was digested for 30 min in 75 µl alkaline lysis reagent (25 mM NaOH, 0.2 mM disodium EDTA, pH 12) at 95 °C. Samples were then cooled to 4 °C, and 75 µl neutralization reagent (40 mM Tris-HCL, pH 5) were added. Undigested tissue debris were pelleted by centrifugation for 5 min at 16200 g.

In order to distinguish between Wt (+) and Mct8 (ko; -) alleles, Mct8ko-A, Mct8ko-B and Mct8ko-C primers were used. To discriminate between Oatp1c1 Wt, flox (fl) and ko (del) alleles, primers Del For, Del Wt and Del Rev were utilized (Table 2.1). Genomic DNA (2 µl of approx. 50 ng/µl) as described above was used in a 30 µl reaction. The PCR thermal cycle steps were as follows: initialization at 95 °C for 2 min, 30 cycles of: denaturation at 95 °C for 20 sec, annealing at 58 °C for 30 sec, elongation at 72 °C for 1 min 30 sec; final elongation at 72 °C for 2 min. Afterwards, samples were applied to a 2% agarose gel to detect the genotype.

Table 2.1 Genotyping primers

Gene	Accession	Primer	Sequence (5´-3´)	Product size
Mct8	NM_00197.2	Mct8 ko-A	TGTGAGTATATTCACTGACCGTTTG	400 bp (+) 650 bp (-)
		Mct8 ko-B	CAATTCAATGGTCAAAGCAGGACTG	
		Mct8 ko-C	GGGCCAGCTCATTCCTCCCACTCAT	
Oatp1c1	NM_021471.1	Del For	CACTGCCCTGTCCTGTAGGT	700 bp (+) 800 bp (fl) 550 bp (del)
		Del Wt	CATCGCTTGATGAGTGGTCTTG	
		Del Rev	ACCATGTGGTTGTTGGGAAT	

2.3 Chemical compounds

The thyromimetic actions of the following compounds were tested *in vitro* as well as *in vivo*: **Sobetirome** (GC-1; Sob1; 2-[4-[(4-hydroxy-3-propan-2-yl-phenyl)methyl]-3,5-dimethylphenoxy]acetic acid) (Chiellini et al., 1998; Placzek and Scanlan, 2015)) was ordered from Sigma-Aldrich (Order Number: SML1900). **Sobetirome 2** (Sob2; Sob-AM2; 2-(4-(4-hydroxy-3-isopropylbenzyl)-3,5-dimethylphenoxy)-N-methylacetamide (Meinig et al., 2017)); **Sobetirome 3** (Sob-3; (S)-1-aminopropan-2-yl2-(4-(4-hydroxy-3-isopropylbenzyl)-3,5-dimethylphenoxy) acetate (Ferrara et al., 2017)); **Sobetirome 4** (Sob4; 2-(3,5-dibromo-4-((3-isopropyl-4-hydroxyphenyl)methyl)phenoxy)acetic acid (Devereaux et al., 2016)); **Sobetirome 5** (Sob5; JD-20AM (Banerji unpublished)) were synthesized in the Scanlan Laboratory (Oregon, USA) as previously described (Meinig et al., 2017; Ferrara et al., 2017; Devereaux et al., 2016). Sobetirome stock solutions at a concentration of 10 mg/ml were prepared using dimethyl sulfoxide (DMSO, Sigma-Aldrich) as solvent and stored at -20 °C. Working solutions were prepared by diluting the stock-solutions in 0.9% saline.

For cell cultures, working solutions of the Sob compounds were diluted in serum-free medium (see 2.4) to get the needed concentrations of 0.01 nM, 0.1 nM, 1 nM, 10 nM and 100 nM.
For treatment of mice with sobetiromes animals were injected subcutaneously with 2 µl/g body weight (bw) of the desired drug at final dose of 100 ng/g bw. Control animals received saline injections. All mice were injected from postnatal day 1 (P1) to P12 once per day between 2-5 pm and body weight was daily recorded in a score sheet. For all studies, at least three animals

per condition were used. Animals were killed five hours after the last injection by isoflurane inhalation followed by intracardial perfusion with 4% paraformaldehyde (PFA, pH: 7.4).

The FAAH-inhibitors PF-3845 (AdooQ Bioscience; cat. no. A11183) and URB937 (Sigma-Aldrich; cat. no. 1357160) were diluted in DMSO for a stock solution of 20 mM and were both further diluted in saline for a concentration of 40 nM. Each inhibitor was added for 14 days (every third day) of cultivation to untreated, 1 nM T3, 10 nM Sob1, 0.1 nM Sob2 or to 1 nM Sob4 and 0.1 nM Sob5 treated cells.

2.4 Cell culture experiments

Mixed cerebellar cultures were prepared as published in earlier studies (Baptista et al., 1994). Briefly, cover slip surfaces were pretreated overnight at 4 °C with high-molecular weight poly-D-lysine (500 µg/ml; P1024, Sigma) and washed three times with distilled water before use. Then, 10 to 15 newborn Wt mice at P0-P1 were killed by decapitation. Cerebella were dissected in ice-cold calcium-magnesium free PBS (CMF-PBS) and meninges were removed under a dissecting microscope. Tissues were collected in ice-cold CMF-PBS and were treated with 1% trypsin in CMF-PBS containing 0.1% DNAse and 0.1% Mg (Worthington, Freehold, NJ) for 3 min at RT before replacing trypsin with DNAse (0.05% in basal medium eagle (BME); Gibco). Cerebella were triturated with three fire-polished Pasteur pipettes of decreasing pore diameter and afterwards centrifuged for 4 min at 410 g. Thereafter, cells were resuspended in 2 ml CMF-PBS containing 1-2 drops DNAse solution (0.5 mg/ml stock) and passed through a 40 µM nylon filter. Subsequently, cells were resuspended in horse serum containing medium and were counted. Finally, 800.000 cells per well were plated in the precoated 24-well culture dishes on coverslips. Cells were allowed to attach overnight, before medium was changed on the next day to complete serum-free medium containing BME (93%), TH- and fatty acid free BSA (10 mg/ml; A-8806, Sigma), glutamax (10 mM; Gibco), glucose (32 mM), penicillin/streptomycin (29 U/ml, Invitrogen) and Sigma-I-1884 supplement (insulin, transferrin, selenite) including the tested compounds of interest. Every three to four days, medium was replaced during a culture period of 14 days. Afterwards, cells were fixed with 4% paraformaldehyde in PBS for 60 min at RT and immunostained as described in 2.6. All experiments were performed at least three times.

In order to visualize PC in primary cultures, cells were immunostained with a rabbit polyclonal antibody against calbindin d28K (1:2000) followed by incubation with an Alexa Fluor 555-labeled goat anti-rabbit secondary antibody. For staining of all GABAergic interneurons a

mouse monoclonal antibody recognizing PV was used (1:1000) and as secondary Alexa Fluor 488-labeled goat anti-mouse antibody. Calbindin- and PV+ cells were randomly selected (15 cells per well, at least 2 wells per treatment and culture) and photographed.

2.5 Immunofluorescence analysis of murine brain sections

Whole brains were removed, post-fixed in 4% PFA in phosphate-buffered saline (PBS) for 24 h at 4 °C and thereafter washed three times in 1x PBS (10 min each). Finally, brains were stored in PBS containing 0.4% sodiumazide at 4 °C until further processing. Fixed forebrains were sectioned coronally on a vibratome (Thermo Scientific Microm HM 650 Vibration microtome) at a thickness of 50 µm. Cerebella were cut sagitally with the same thickness and all sections were stored in PBS containing 0.4% sodiumazide at 4 °C.

For visualizing the proteins of interest in forebrain and cerebellum, vibratome sections were blocked and permeabilized in 1x PBS containing 0.2% Triton X-100 and 10% normal goat serum (NGS, Sigma-Aldrich) for 1 h at RT and then incubated overnight at 4 °C with primary antibodies (Table 2.2) freshly diluted in PBS containing 0.2% Triton X-100 and 1% NGS.

To visualize the GABAergic interneurons, brain sections were incubated with anti-Parvalbumin antibody to count the positive stained cell number. For the visualization of the myelination, brain sections were stained against myelin basic protein (MBP) to measure the integrated intensity and to assess the state of the PC dendritic development, cerebellum sections were incubated with a monoclonal mouse anti-Calbindin d28k antibody to analyze the thickness of the molecular layer at the primary fissure between anterior and posterior lobe.

Subsequently, sections were washed three times in 1x PBS before incubated for 1 h at RT with an Alexa Fluor-labeled secondary antibody (Alexa Fluor 488 or Alexa Fluor 555, Life Technologies) and Hoechst33258 (MolecularProbes, Leiden, the Netherlands) to label cell nuclei. Finally, sections were mounted in water-based fluoromount media and photographed using Zeiss AxioObserver Z1 ApoTome microscope (Zeiss, Germany). In each experimental group, three to five mice were included for the immunhistochemical analysis.

Table 2.2 Information of primary and secondary antibodies

Name of antibody	Host/Type	Company	Catalog number	Dilution
anti-Parvalbumin	mouse monoclonal	Millipore, Billerica, USA	MAB1572	1:1000
anti-Myelin Basic Protein	rat monoclonal	Millipore, Billerica, USA	MAB386	1:300
anti-Calbindin d28k	rabbit polyclonal	Swant, MArly, Switzerland	CB38	1:500
anti-Calretinin	rabbit polyclonal	Swant	CR7697	1:2000
anti-GAD67	mouse monoclonal	Millipore	MAB5406	1:1000
anti-NeuN	mouse monoclonal	Millipore	MAB377	1:500
anti-mouse, Alexa Fluor ®555 conjugate	goat polyclonal	Thermo Fisher, Massachusetts, USA	A21425	1:1000
anti-rat, Alexa Fluor ®555 conjugate	goat polyclonal	Thermo Fisher, Massachusetts, USA	A21434	1:1000

2.6 Quantification

All quantifications were conducted with ImageJ. In cerebellar vibratom sections, thickness of the molecular layer was determined by measuring the PC dendritic dimension on three consecutive sections from each animal (at least n = 3 per genotype). For determining the number of PV+ GABAergic interneurons in the cerebral cortex, immunopositive cells were counted on three consecutive sections from each animal using the multipoint tool of ImageJ and normalized to the analyzed cortical area. MBP staining in the corpus callosum (CC) was calculated by framing the respective area, followed by measuring the integrated density.

To quantify PC dendritic outgrowth *in vitro*, the total area covered by the soma and the dendritic tree of around 20 randomly selected cells of each condition were framed and the perimeter (outline of the cell and dendritic branches) was measured. All values were normalized to the control (0 nM T3), set as 100%. For determining the number of PV- expressing neurons, five pictures for each condition were selected and the number of PV+ cells normalized to the total

cell number was determined. Again, number of cells found in cultures with 0 nM T3 was defined as 100 %.

2.7 Statistical analysis

For the statistical analysis of the results GraphPad Prism 7.04 was used. All values were presented as mean ± SEM. For comparisons between different groups nonparametric One-Way ANOVA with a following Tukey´s post hoc test were performed and differences were assessed as significant if the P value reached below 0.05 (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

3 Results

3.1 Effects of Thyroid hormone analogs in primary cerebellar culture

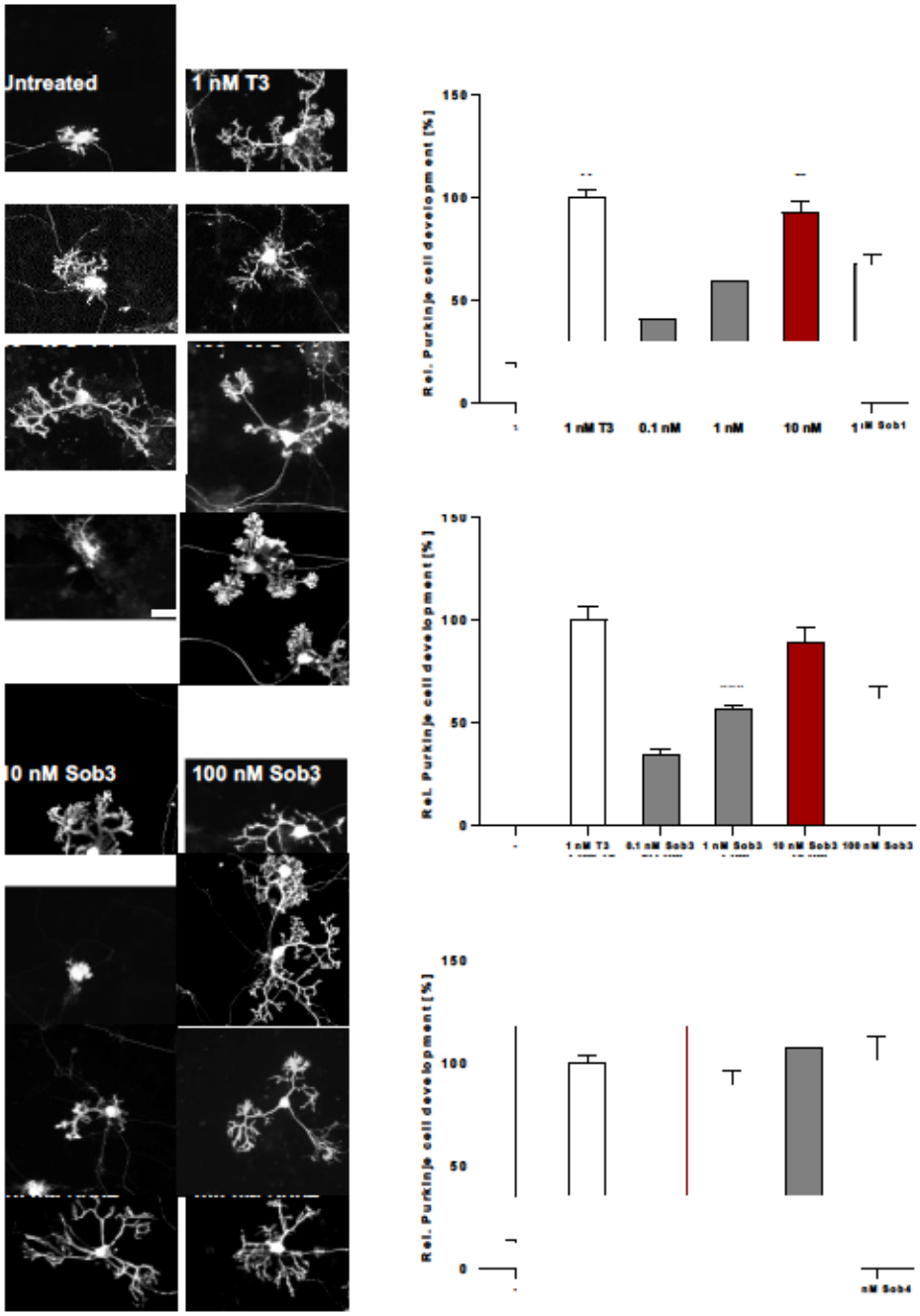
TH are essential for normal CNS development. As an example, the development of the cerebellum that regulates motor function and coordination is highly sensitive to TH deprivation (Oppenheimer and Schwartz, 1997). Especially the differentiation of PC, some of the largest neurons in the brain, is strongly retarded in the absence of TH. Thereby, these cells show abnormal organization of the PC trunk, delayed outgrowth and arborization of the dendritic tree as well as a reduced spine number. It has been reported, that the presence of 1 nM T3, a concentration equal to T3 serum levels in adult mice, exert the most pronounced effects on PC dendritogenesis with fully grown spines and branches (Heuer and Mason, 2003). Further, it has been observed that the differentiation of cerebellar GABAergic interneuron precursors which migrate from the white matter into the cerebellar cortex to differentiate into Golgi, basket and stellate cells, are also compromised by TH deprivation (Manzano et al., 2007).

In a first approach the TH analogs Sob1 - Sob5 were tested in murine primary cerebellar culture to analyze their TH-like effect. For that purpose, primary cell cultures were prepared from cerebellar cortex of neonatal Wt mice according to protocols published in Baptista et al., 1994 and Hatten et al., 1998. Cells were cultured on serum-free conditions in the presence or absence of 1 nM T3 as a control or with sobetiromes at different concentrations (0.01 nM, 0.1 nM, 1 nM, 10 nM or 100 nM). After 14 days in culture, cells were immunostained with an anti-calbindin antibody to visualize PC and with anti-Parvalbumin antibody to visualize PV-expressing GABAergic interneurons as both cell types are strongly dependent on the presence of T3 (Vincent et al., 1982; Legrand, 1984; Nicholson and Altman, 1972).

To quantify the dimensions of PC outgrowth, PC area covered by the soma and dendritic tree was traced with ImageJ. To analyze the number of PV+ cells, randomly selected areas of cultures were photographed and counted.

3.1.1 Effects of sobetiromes on Purkinje cell development

In the absence of TH, PC do not develop a fully developed dendritic tree whereas the strongest effect on dendritogenesis is obtained in the presence of 1 nM T3 (Heuer and Mason, 2003). Similar stimulating effects on arborization were observed in the presence of 10 nM Sob1 (Fig.3.1A, B) and 10 nM of Sob3 (Fig.3.1C, D). Sob4 (Fig.3.1E, F) showed T3-like effects already at a concentration of 1 nM, while Sob2 (Fig.3.1G, H) and Sob5 (Fig.3.1I, J) were similarly effective at a concentration of 0.1 nM. These results indicate that sobetiromes are effective substitutes for T3 *in vitro* since PC respond to a specific dose of sobetiromes with an increase in dendritic length.



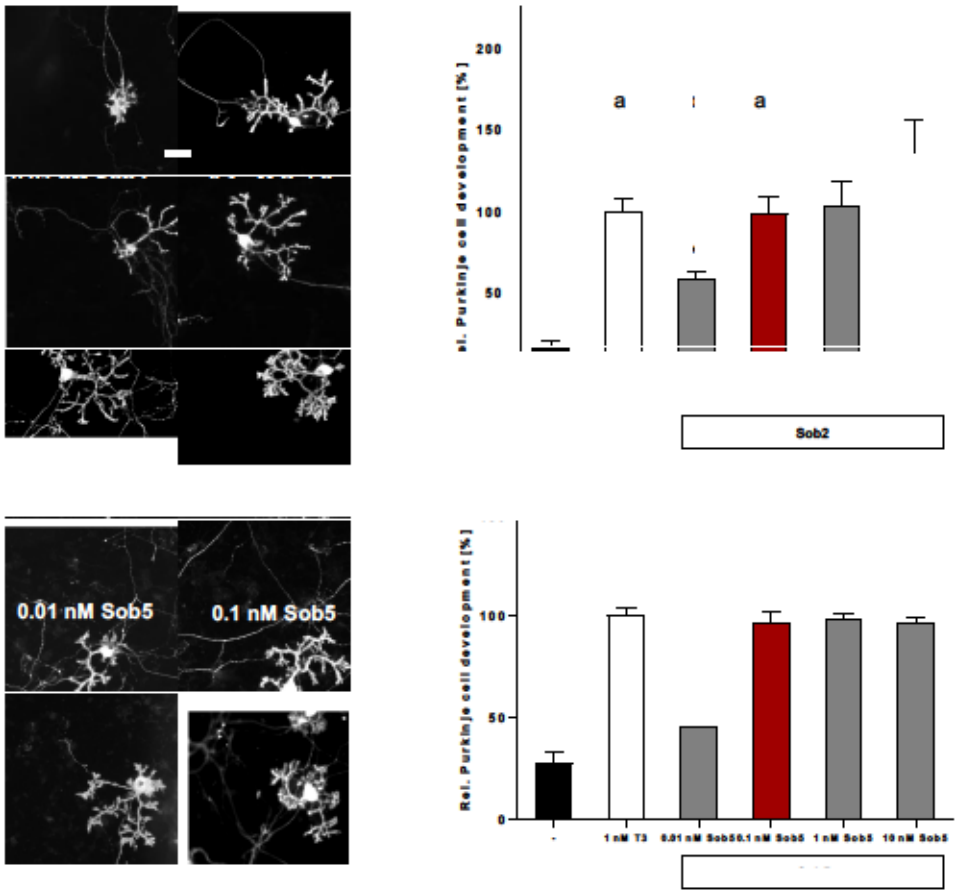
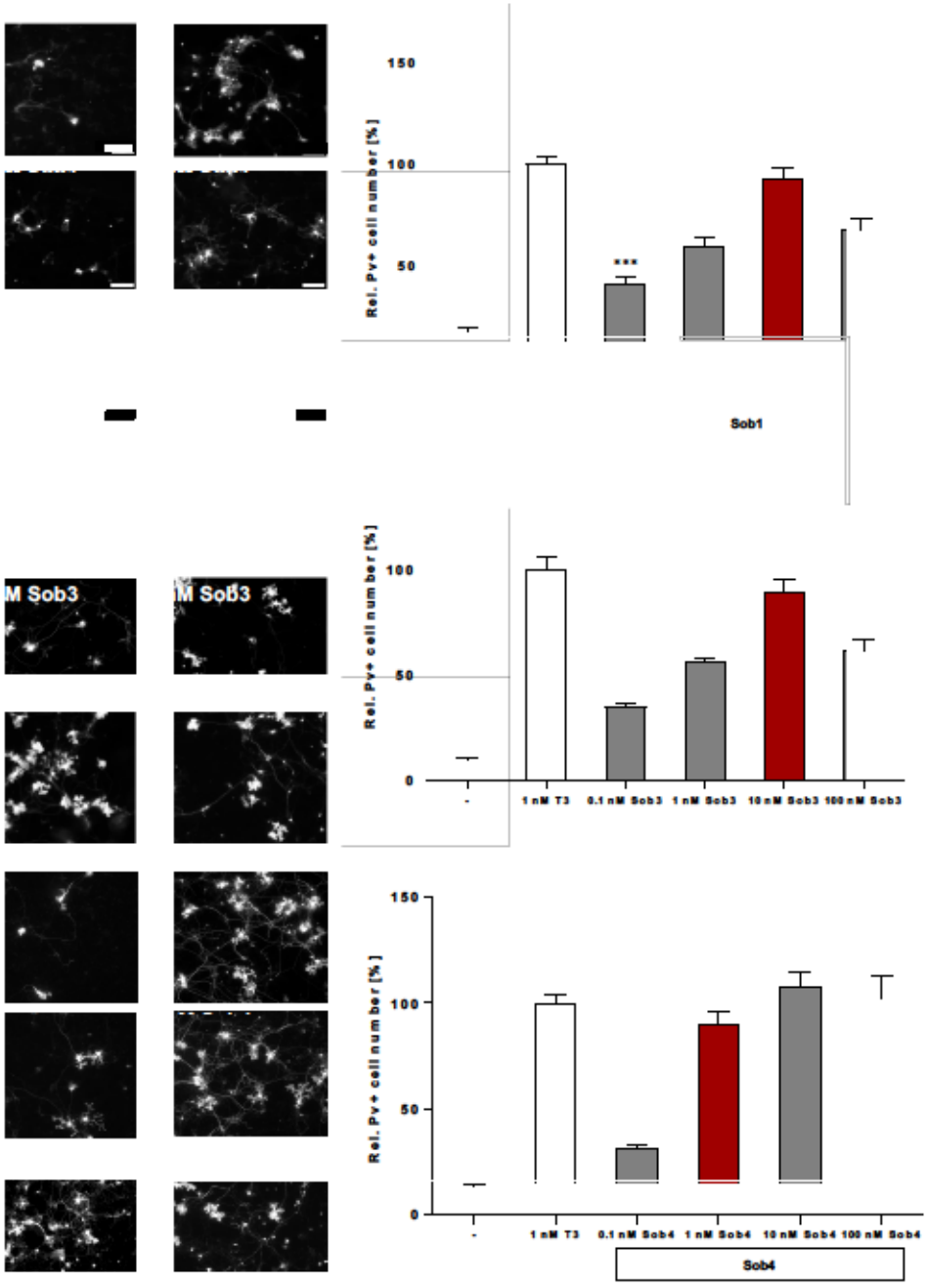


Fig.3.1 Effects of sobetiromes 1-5 on cerebellar PC dendritogenesis *in vitro*. Mixed cerebellar cultures from neonatal Wt mice were cultured for 14 days in the absence or presence of T3 or Sob1 - Sob5. Development of PC was visualized by calbindin D28k staining (A, C, E, G, I). PC perimeter was quantified and normalized to the perimeter of PC treated with 1 nM T3. Treatment of the cultures with 1 nM T3 resulted in pronounced formation of dendrites as shown by an almost 4-fold increase in PC perimeter. The same stimulating effect was achieved by adding 10 nM Sob1 (B), 10 nM Sob3 (D) 1 nM Sob4 (F) 0.1 nM Sob2 (H) and 0.1 nM Sob5 (J) to the cultures. ***P < .001 for comparison with 1 nM T3 treated cells; a (***), b (**) for comparison with untreated cells. Each column and error bar represent the mean ± SEM from three independent experiments. Red bars represent lowest Sob concentration that shows the best T3-like effect *in vitro*. Scale bar, 20 µm.

3.1.2 Effects of sobetiromes on parvalbumin positive cells

Apart from PV-expressing PC, cerebellar cultures contain also PV-expressing GABAergic interneurons such as stellate, basket and Golgi cells. Number of PV+ cells was counted in cultures treated with different concentrations of the sobetiromes (0.01 nM, 0.1 nM, 1 nM, 10 nM and 100 nM). The number of PV+ cells was strongly reduced in the absence of T3 (Fig.3.2). Application of Sob1 (A, B) and Sob3 (C, D) normalized the number of PV+ cells with a concentration of 10 nM reaching T3-like effects. For Sob4, a normal number of PV+ cells were obtained at a concentration of 1 nM (E, F), while Sob2 and Sob5 reached T3-like effects with just 0.1 nM.


M Sob3
M Sob3
Rel. Pv+ cell number [%]
150
100
50
***
Sob1
Rel. Pv+ cell number [%]
100
50
0
-
1 nM T3
0.1 nM Sob3
1 nM Sob3
10 nM Sob3
100 nM Sob3
Rel. Pv+ cell number [%]
150
100
50
0
-
1 nM T3
0.1 nM Sob4
1 nM Sob4
10 nM Sob4
100 nM Sob4
Sob4

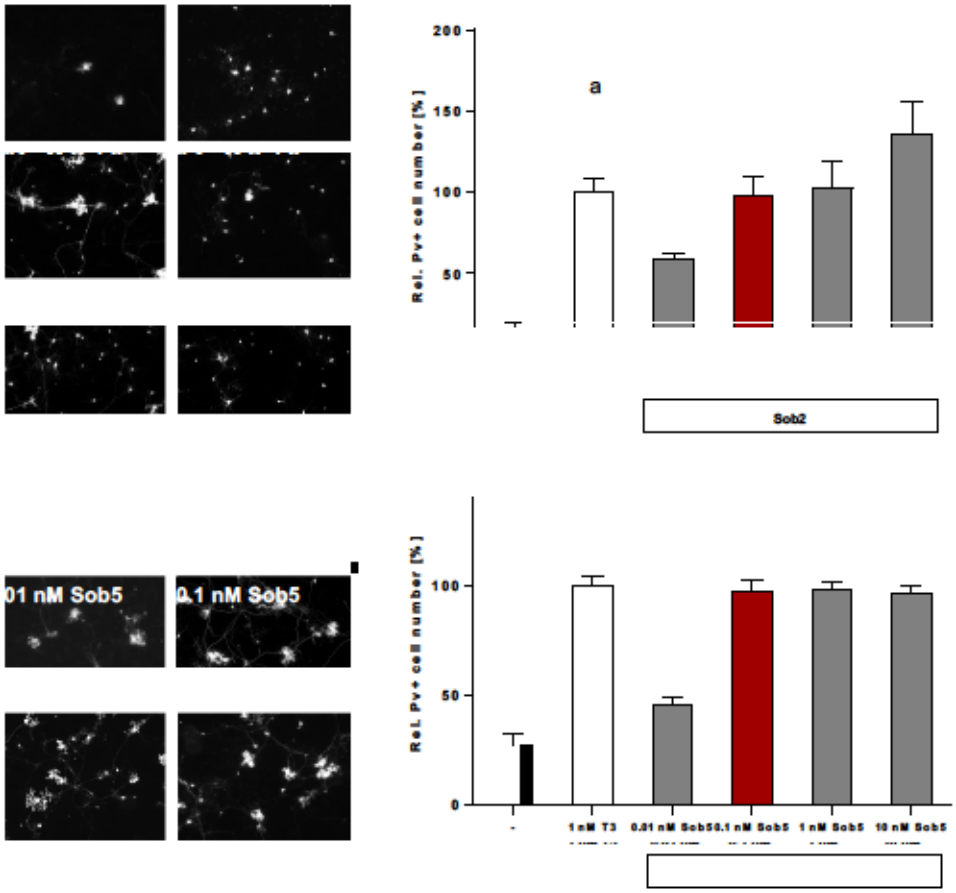


Fig.3.2 Effects of sobetiromes 1-5 on PV+ cells *in vitro*. Mixed cerebellar cultures from neonatal Wt mice were cultured for 14 days in the absence or presence of T3 or Sob1 – Sob5. PV+ cells were visualized by immunofluorescence stainings using an anti-Parvalbumin antibody. Number of PV+ cells were quantified and normalized to the number of cells treated with 1 nM T3 only. Treatment of the cultures with 1 nM T3 resulted in a 6-fold increase in the number of PV+ cell number. The same stimulating effect was achieved by adding 10 nM Sob1 (A, B), 10 nM Sob3 (C, D), 1 nM Sob4 (E, F), 0.1 nM Sob2 (G, H) and 0.1 nM Sob5 (I, J) to the cultures. *** $P < .001$, * $P < .05$ for comparison with 1 nM T3 treated cells; a (***), b (**) for comparison with untreated cells. Each column and error bar represent the mean ± SEM from three independent experiments. Red bars represent lowest Sob concentration that shows the best T3-like effect *in vitro*. Scale bar, 40 µm.

These data show that all sobetiromes are able to stimulate PC development and PV+ expressing cells *in vitro*, but with different efficacy. Sob2 and Sob5 seem to be the most promising sobetirome compounds in culture.

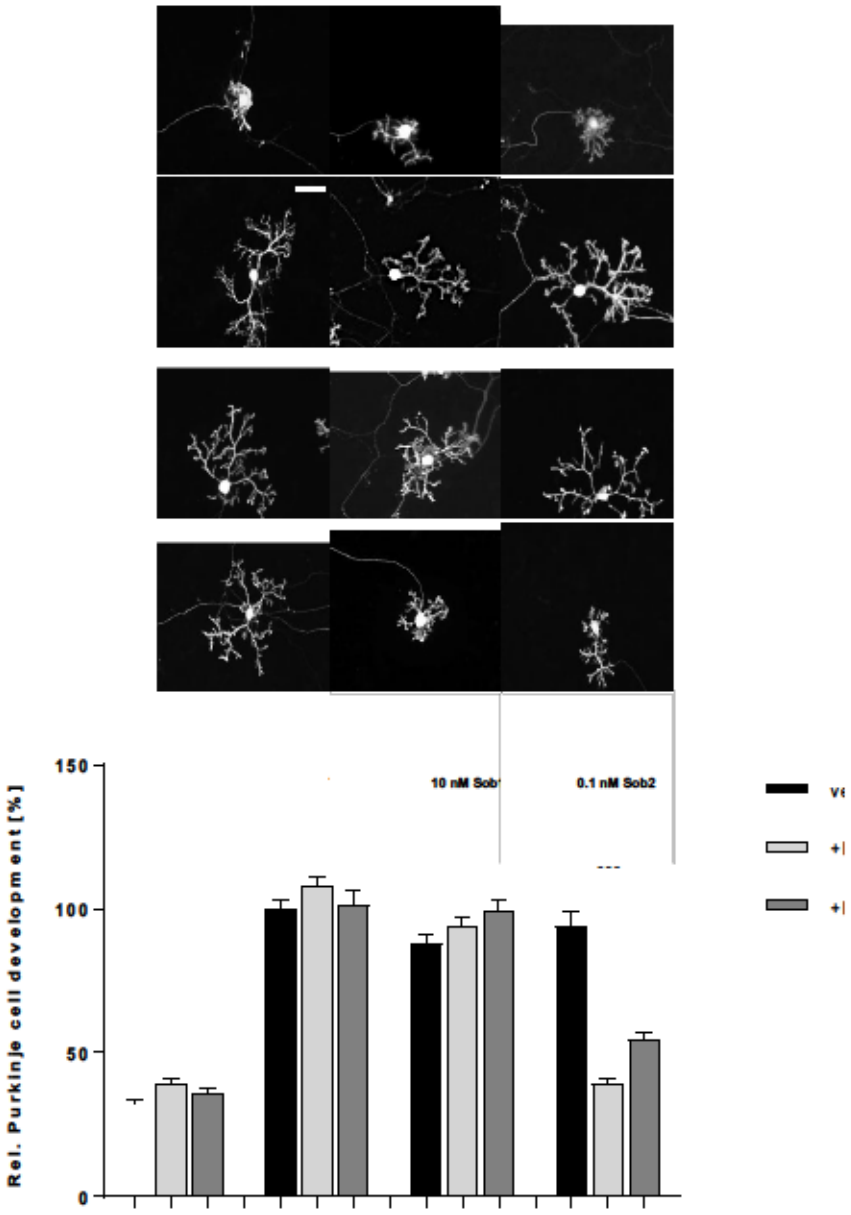
3.2 Sob2 and Sob5 as Fatty Acid Amide Hydrolase substrates

It has been shown that sobetirome does distribute to the CNS, but the fraction of the administered dose that reaches the CNS is very low compared to other approved CNS drugs (Doran et al., 2005). Therefore, strategies to increase CNS distribution of sobetirome while concomitantly decreasing peripheral exposure were examined by creating new agents with improved CNS distribution profiles. These efforts have involved the creation of prodrugs of sobetirome (Sob2 and Sob5) that mask sobetirome in circulation and peripheral tissues, but liberate sobetirome upon hydrolysis in the CNS. Due to their structural similarity to anandamide, one of the endogenous cannabinoid substrates for FAAH, it has been hypothesized that FAAH is responsible for cleavage of this prodrugs to produce the active sobetiromes (Meinig et al., 2017).

In order to verify this prodrug hypothesis, two FAAH-inhibitors (Inhibitor 1 = PF-3845 and Inhibitor 2 = URB-973) were tested in primary cerebellar culture (Ahn et al., 2009; Sasso et al., 2012). Each inhibitor (40 µM) was added for 14 days (every third day) of cultivation to untreated, 1 nM T3, 10 nM Sob1, 0.1 nM Sob2 or to 1 nM Sob4 and 0.1 nM Sob5 treated cells. PC outgrowth and the number of PV+ cells were visualized and quantified.

3.2.1 Effects of FAAH inhibitors on Purkinje cell development

Addition of inhibitor 1 (Inh.1) or inhibitor 2 (Inh.2) did not change the PC development of untreated, 1 nM T3 or 10 nM Sob1 treated cells (Fig.3.3A, B) indicating that the application of these FAAH inhibitors does not interfere with T3-induced PC dendritogenesis. However, in cultures treated with the Sob-prodrug Sob2 a significant decrease in the PC outgrowth were achieved by FAAH inhibition (60% decrease with Inh.1 and 40% decrease with Inh.2).
Similar effects were observed when Inh.1 or Inh.2 were added to the Sob4-prodrug Sob5 (Fig. 3.3C, D). Here the PC outgrowth was abolished up to 60% with Inh.1 and up to 50% with Inh.2 compared to the control.


10 nM Sob
0.1 nM Sob2
Rel. Purkinje cell development [%]
150
100
50
0

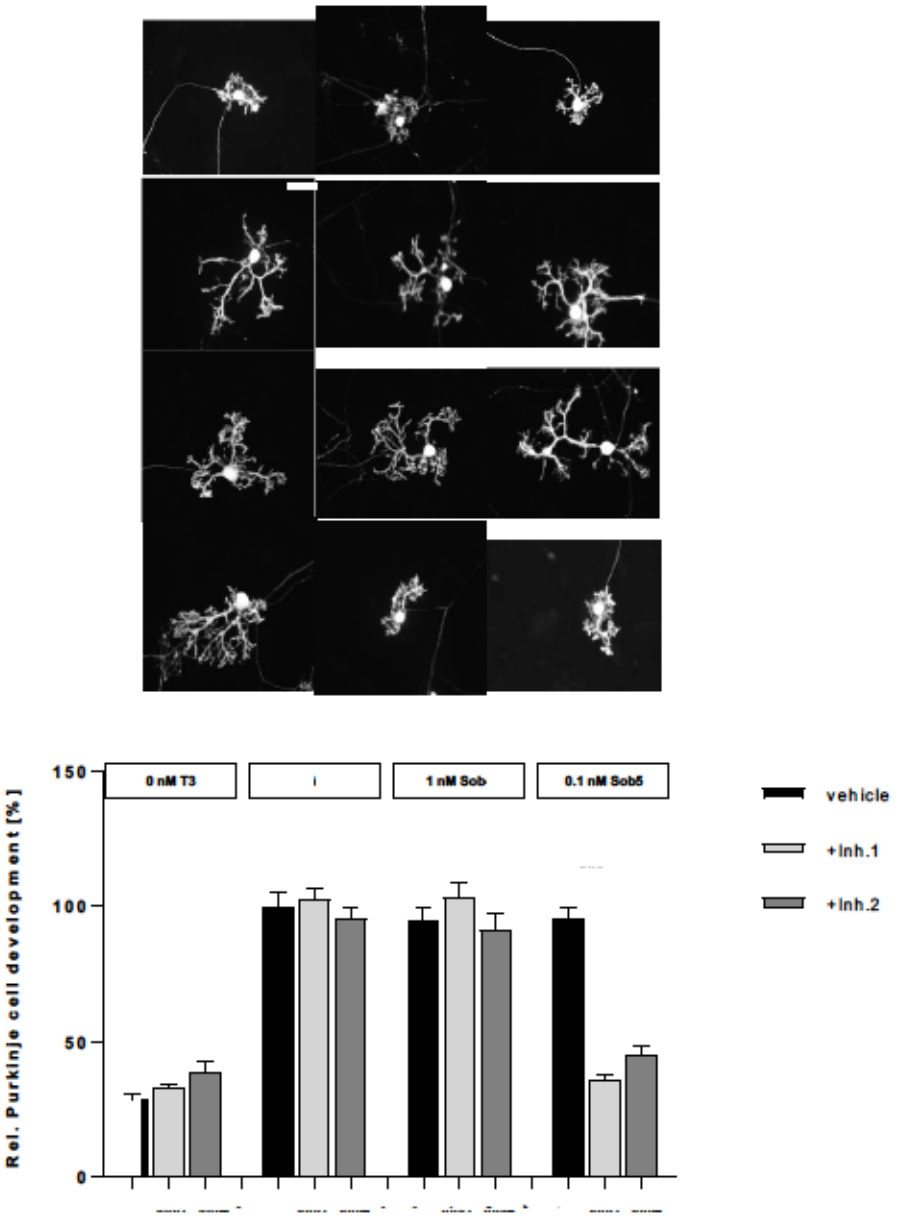
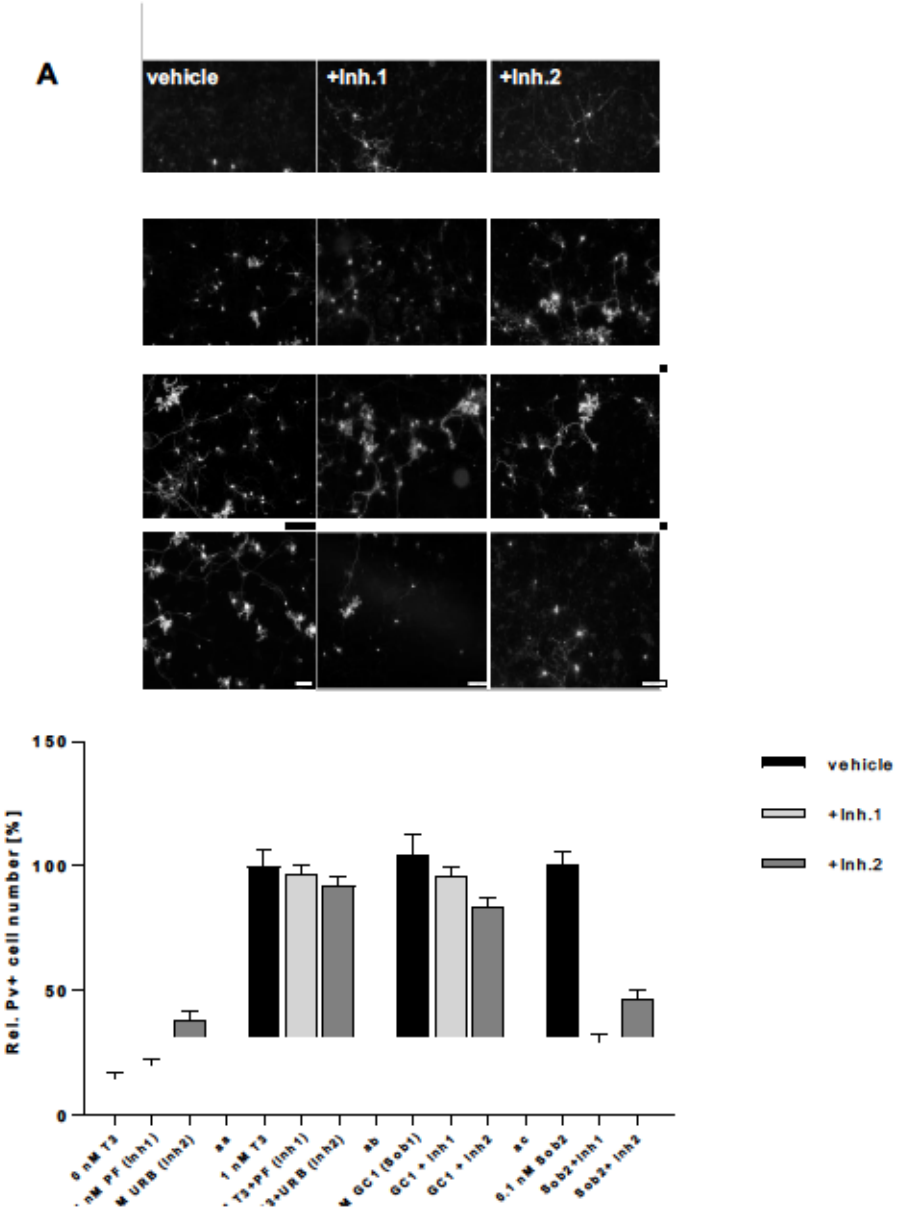


Fig.3.3 Effects of FAAH inhibitors on cerebellar PC dendritogenesis. Mixed cerebellar cells from neonatal Wt mice were cultured for 14 days under different conditions: 0 nM T3, 1 nM T3, 10 nM Sob1 and 0.1 nM of its prodrug Sob2 (A, B) or 1 nM Sob4 and 0.1 nM of its prodrug Sob5 (C, D). In addition, cultures were treated with Inh.1 or Inh.2 (40 µM). Development of PC was visualized by calbindin staining, quantified and normalized to the perimeter of PC treated with 1 nM T3 only. Both, Inh.1 and Inh.2 abolished the compound-induced PC development just in combination with the prodrugs Sob2 and Sob5. *** $P < .001$. Each column and error bar represent the mean ± SEM from three independent experiments. Scale bar, 40 µm.

3.2.2 Effects of FAAH inhibitors on parvalbumin positive cells

Addition of Inh.1 or Inh.2 did not change the PV+ cell number of untreated cells, 1 nM T3 or 10 nM Sob1 treated cells. A significant decrease of 70% could be detected in the PV+ cell number upon adding Inh.1 and a 50% decrease upon adding Inh.2 to the prodrug Sob2 (Fig.3.4A, B).

Similar effects were observed when Inh.1 or Inh.2 was added to prodrug Sob5 (Fig.3.4C, D). Here the number of PV+ cells was abolished up to 70 % (Inh.1) and 60% (Inh.2) compared to the control without inhibitor.



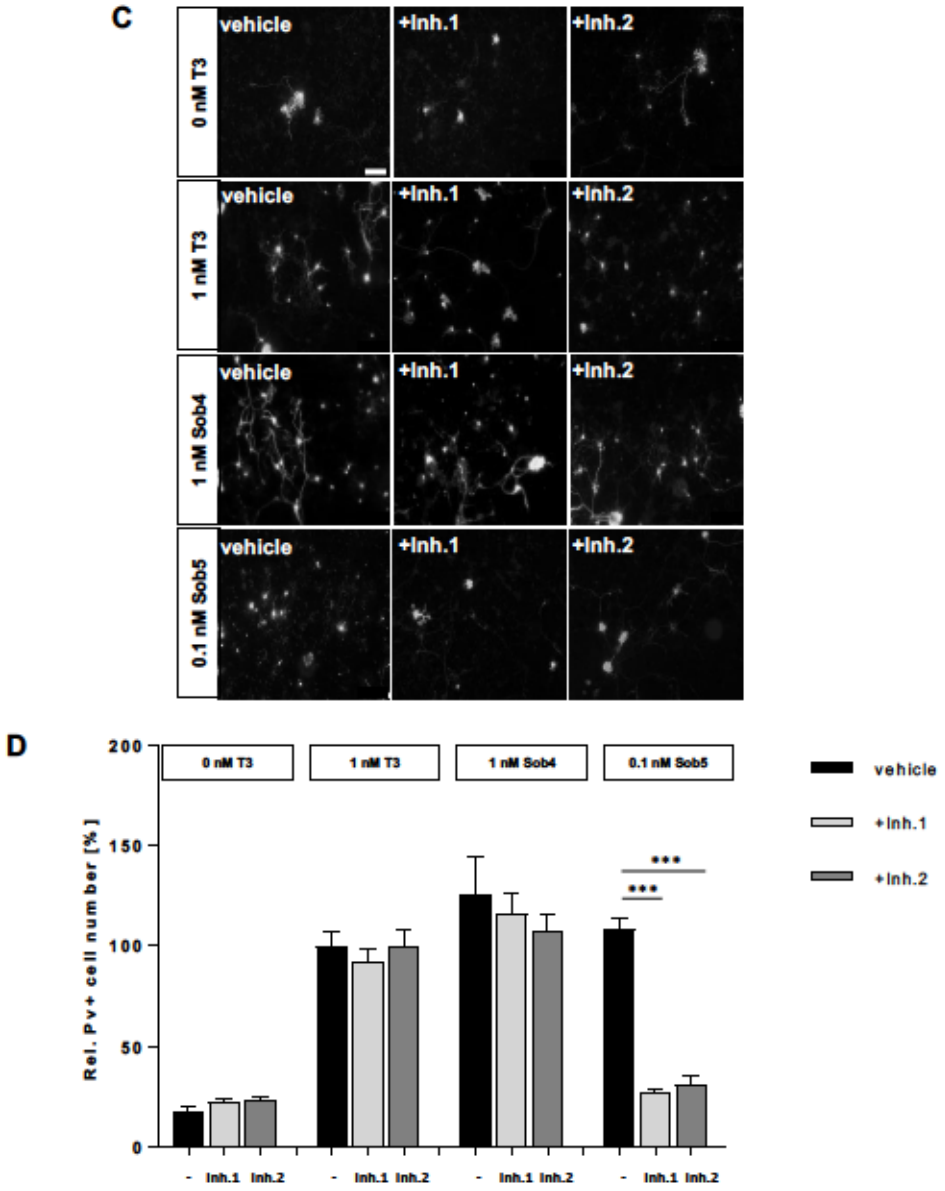


Fig.3.4 Effects of FAAH inhibitors on PV+ cell number. Mixed cerebellar cultures from neonatal Wt mice were cultured for 14 days in the presence of 0 nM T3, 1 nM T3, 10 nM Sob1 and 0.1 nM of its prodrug Sob2 (A, B) or 1 nM Sob4 and its prodrug 0.1 nM Sob5 (C, D). In addition, cultures were treated withe Inh.1 or Inh.2 (40 µM). Number of PV+ cells in the respective cultures were quantified and normalized to the number of cells treated with 1 nM T3 only. Both, Inh.1 and Inh.2 reduced the compound-induced cell number just in combination with the prodrugs Sob2 and Sob5. *** $P < .001$. Each column and error bar represent the mean ± SEM. Scale bar, 40 µm.

These data demonstrate that FAAH inhibitors do not interfere with T3-induced action but impede the Sob2 and Sob5 prodrug-induced PC outgrowth as well as the number of PV+ cells.

3.3 Effect of TH analogs in the brain during early postnatal development

Since the *in vitro* experiments showed positive effects of the sobetirome compounds in cerebellar PC outgrowth as well as in the number of PV+ neurons, the next part of this study was to determine their effect *in vivo*.

Thus, the aim of this part was to analyze the efficacy of Sob1 - Sob5, in ameliorating neuronal differention in of M/O dko mice. For that purpose, M/O dko mice were genotyped after birth and treated with one of the sobetiromes between postnatal day P1 and P12 similar to previous studies that were conducted for assessing the thyromimetic action of the TH analog Triac (Kersseboom et al., 2014).

3.3.1 Effects of sobetiromes on cerebellar Purkinje cell development

In rodents cerebellar PC outgrowth takes place within the first three postnatal weeks and is also *in vivo* highly dependent on proper TH supply (Legrand, 1984). In order to determine the effect of the sobetiromes on PC development *in vivo*, cerebellar sections from P12 animals were immunostained with an antibody against the calbindin D28. For quantification, only pictures of lobule V in the anterior domain of the cerebellum were analyzed and the thickness of the molecular layer (ML) that represents the dimension of the PC dendritic tree, was measured. As already demonstrated previously (Mayerl et al., 2014), PC in M/O dko mice displayed a stunted dendritic arborization with a 50% reduction in molecular layer thickness compared to the respective Wt controls (Fig.3.5A). Treatment of M/O dko mice with sobetiromes at a dose of 100 ng/g bw improved the dendritogenesis of PC. No morphological differences between the sobetirome treated and the Wt mice could be detected (Fig.3.5B). These results indicate a complete normalization of this parameter.

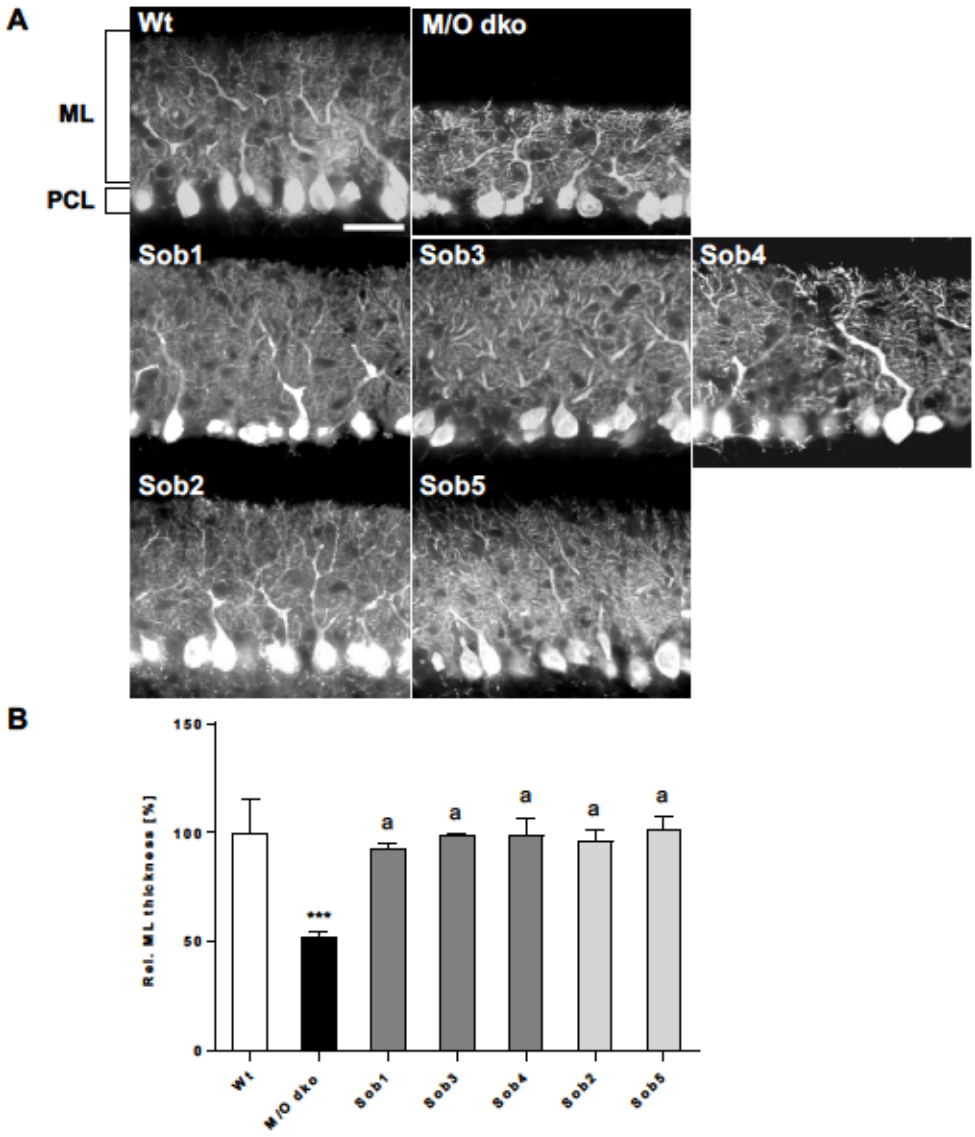


Fig.3.5 Effects of sobetiromes treatment on cerebellar Purkinje cell development *in vivo*. Staining of cerebellar sagittal sections from P12 animals with an anti-calbindin antibody revealed a strongly decreased outgrowth of the PC dendritic tree in the M/O dko mouse, which was rescued by Sob1 - Sob5 treatment (A). PC dimension was measured in order to quantify the thickness of the molecular layer (ML). A 50% reduction in the thickness of ML was observed in untreated M/O dko mice, whereas Sob treated M/O dko mice showed similar thickness as Wt animals (100 ng/g bw) (B). Compared to Wt: ***P<0.001; compared to M/O dko: a=***. n=3. Scale bar, 30 µm.

3.3.2 Effects of sobetiromes on parvalbumin-expressing cerebellar interneurons

Previous studies on M/O dko mice have revealed a delayed differentiation of GABAergic inhibitory interneurons, evidenced by a reduced number of PV+ cells in the somatosensory cortex (Mayerl et al., 2014). As the cell culture experiments described in 3.1.2 reveald a profound impact on T3 on cerebellar PV+ cell number, sagittal cerebellar sections from P12 old animals were immunostained with an anti-Parvalbumin antibody and number of positive cells (without including PC) were observed for a qualitative statetment. The PV+ cell number (white arrows) seemed to be reduced in M/O dko mice compared to the Wt littermates while the PV+ cell number seemed to be normalized with Sob1, Sob4, Sob2 and Sob5. Sob3 appeared to just partially restore the PV+ cell number (Fig.3.6). For a quantitative analysis more animals have to be included.

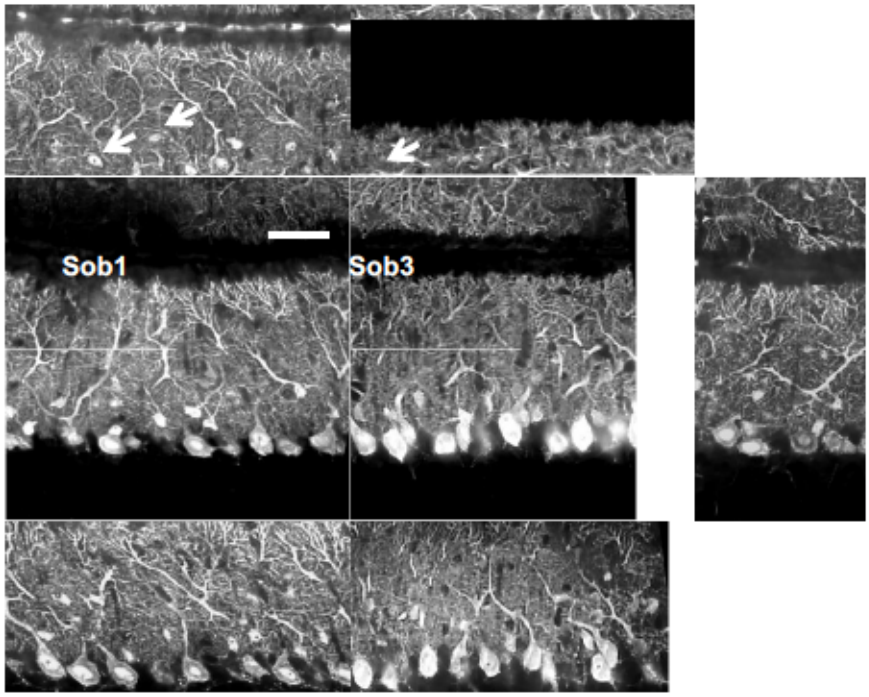
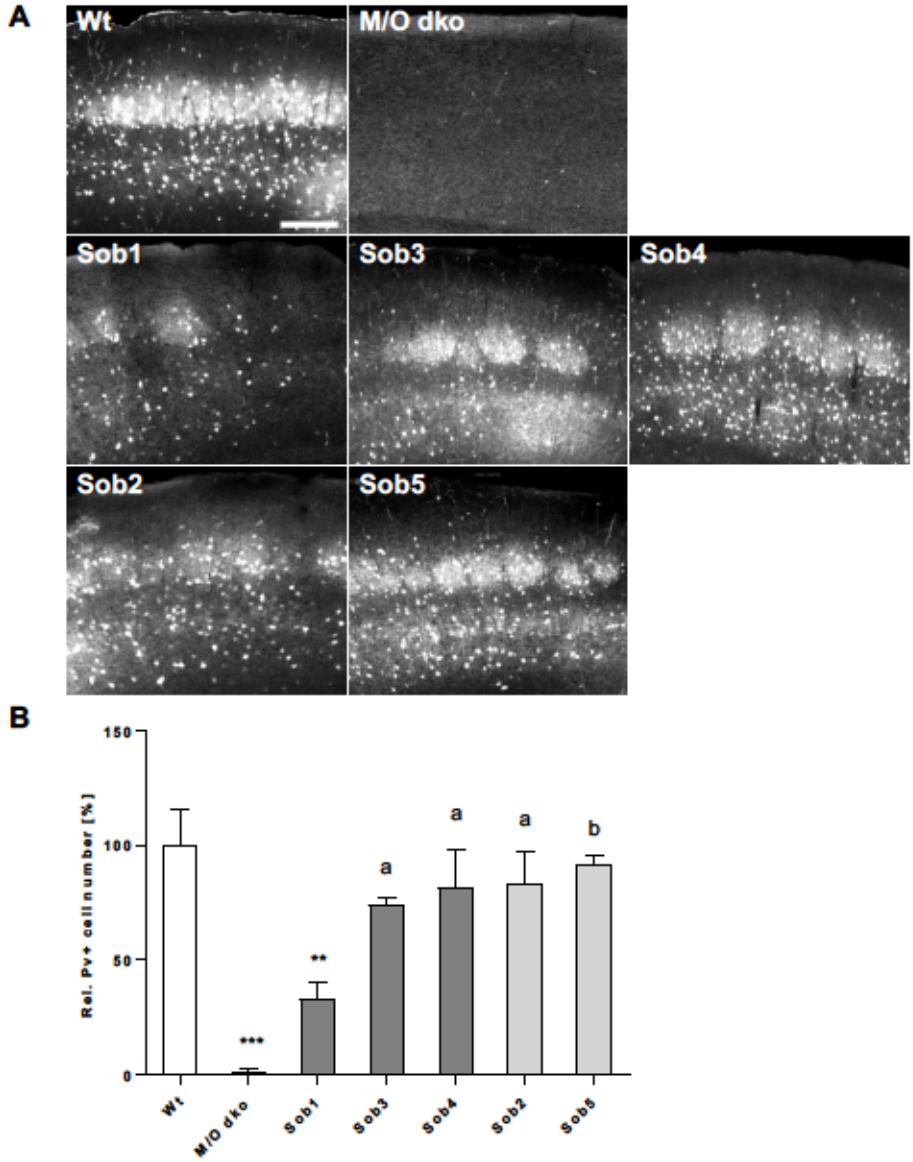


Fig.3.6 Effects of sobetirome 1-5 treatment on parvalbumin-expressing cells in the cerebellum. Immunofluorescence staining of cerebellar sagittal sections of P12 animals with an anti-Parvalbumin antibody revealed a decreased PV+ cell number (white arrows) in M/O dko mouse, which was rescued by Sob1 - Sob5 treatment with a concentration of 100 ng/g bw. n=3. Scale bar, 30 µm.

These data demonstrate that all sobetiromes are able to normalize the PC outgrowth as well as the number of PV+ interneurons in the cerebellar cortex of M/O dko mice.

Analysis of PV-expressing interneurons in the cerebral cortex

Apart from a disturbed cerebellar development, M/O dko mice exhibit pronounced alterations in the cerebral cortex (Mayerl et al., 2014). In particular PV immunoreactivity in the somatosensory cortex has been showen do be dimished in M/O dko mice. In order to evaluate the impact of TH analogs on this parameter, coronal forebrain sections from P12 animals were immunostained with an anti-Parvalbumin antibody, and positively stained PV cells were counted in the primary somatosensory cortex (Fig.3.7A) and in the retrosplenial cortex (Fig.3.7C). In untreated M/O dko mice, the PV+ cell population was significantly decreased to less than 10% compared to control animals (Fig.3.7B, D). This reduction could be partially prevented by treating the animals with Sob1 and Sob3 (25% - 65%), whereas application of Sob2, Sob4 and Sob5 resulted in a greater improvement of these numbers (80% - 90%). Similar effects were detectable in the retrosplenial cortex (Fig.3.7C). While Sob1 and Sob3 could only partially normalize the number of PV+ cells, Sob4, Sob2 and Sob5 normalized this parameter almost to the level of Wt controls.



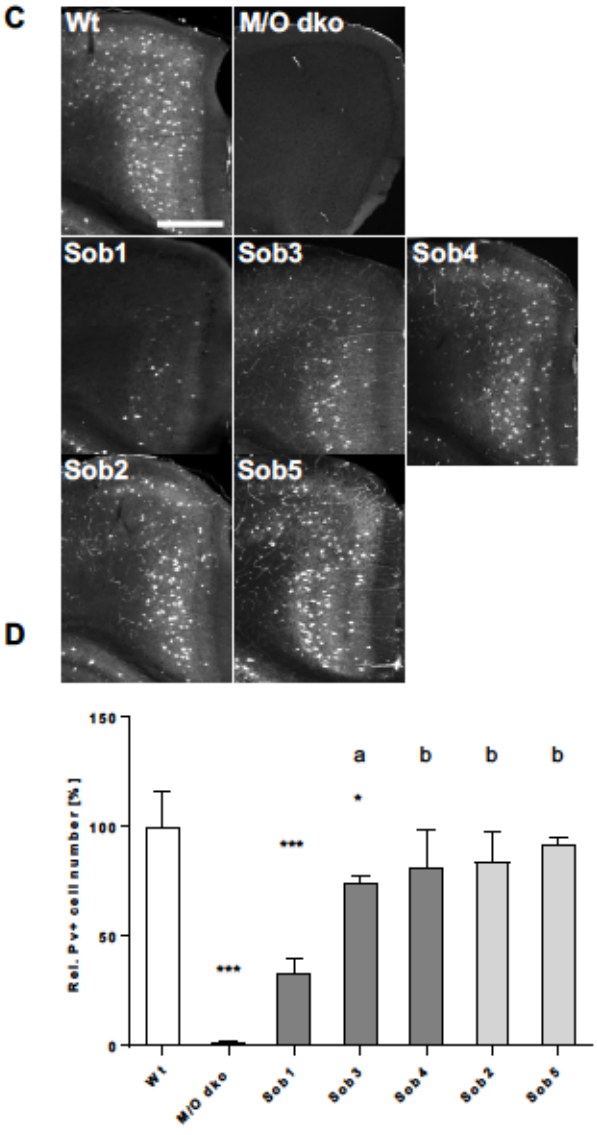


Fig.3.7 Effects of sobetirome treatment on number of parvalbumin-expressing cells in the primary somatosensory and in the retrosplenial cortex. Coronal sections derived from P12 old animals were immunostained with anti-Parvalbumin antibody. Almost no positively stained cells were present in the primary somatosensory cortex (A) or in the retrosplenial cortex (C) of untreated M/O dko mice. Application of Sob compounds could restore this parameter to a different extend ranging from weak (Sob1) to moderate (Sob3) up to an almost full normalization (Sob2, Sob4, Sob5). Compared to Wt: *P<0.05, **P<0.01, ***P<0.001; compared to M/O dko: a(**), b(***). n=3. Scale bar for somatosensory cortex: 200 µm, and for retrosplenial cortex: 100 µm.

3.3.3 Effects of sobetiromes on calretinin-expressing interneurons

To further evaluate the development of the GABAergic system in the cerebral cortex under Sob treatment, coronal forebrain vibratome sections derived from P12 male mice were immunostained with an antibody against calretinin. Calretinin-positive cell bodies were visible throughout all cortical layers, but especially concentrated in cortical layer II/III (Fig.3.8A). The number of calretinin-positive interneurons was counted and quantified (Fig.3.8B). M/O dko mice showed a significant increase in calretinin-positive cells, while no changes were found between Sob1-5 treated M/O dko mice and Wt animals.

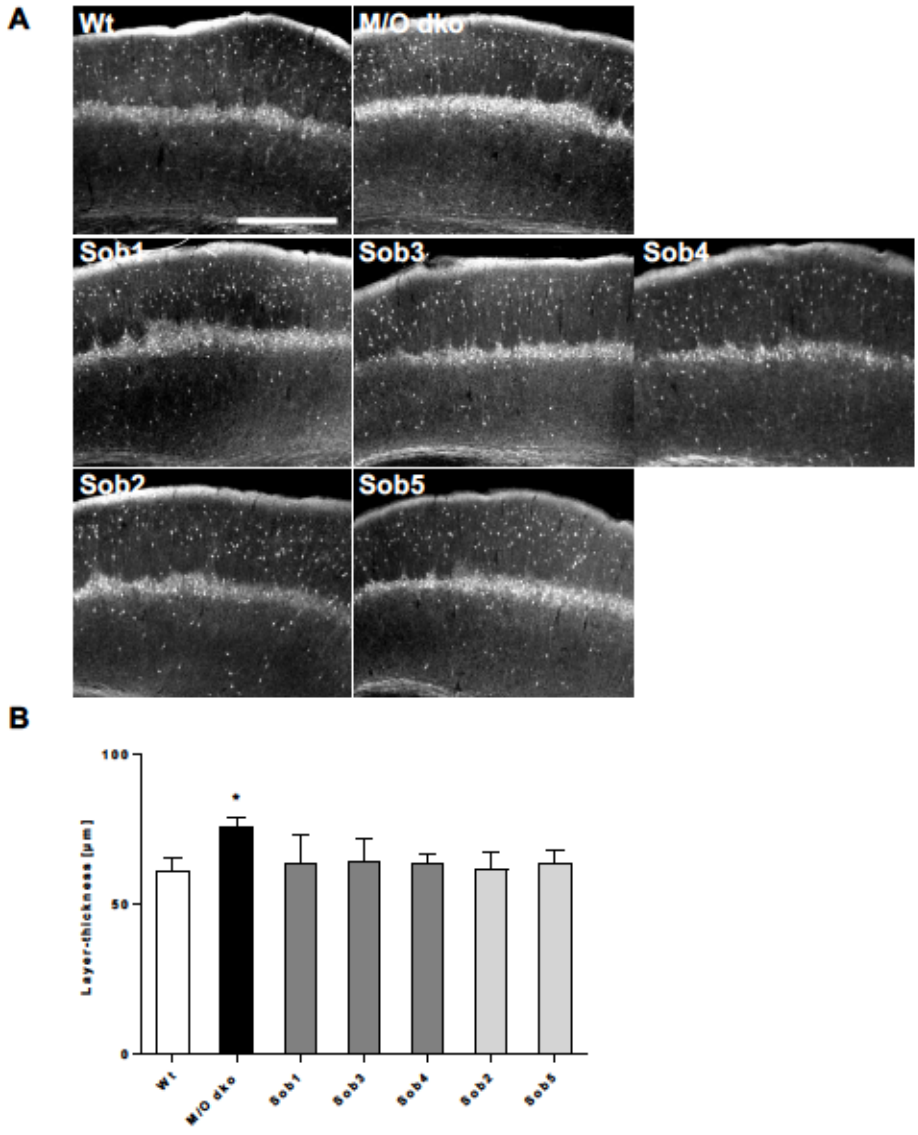


Fig.3.8 Effects of sobetiromes treatment on calretinin expression in the somatosensory cortex. Coronal sections derived from P12 old animals were stained with anti-calretinin antibody. Calretinin immunoreactivity was present throughout all cortical layers, with the highest abundance in the cortical layers II/III. M/O dko mice showed an increased number of calretinin-positive cells. No differences were observed between Sob1 - 5 and Wt. *P<0.05; n=3. Scale bar, 400 µm.

3.3.4 Effects of sobetiromes on GAD67 immunoreactivity

Glutamate decarboxylase 67 (GAD67) that is widely distributed throughout cell bodies, axons and proximal dendrites of neuronal cells, catalyzes the production of GABA (Kaufman et al., 1991). Immunohistochemical analysis of GAD67 in the somatosensory cortex of P12 male Wt mice showed positive signals in all cortical layers with strongest intensities in the distinct barrels (Fig.3.9A). M/O dko mice exhibited highly diminished GAD67 immunoreactivity (~40%). Sob5 showed a relative integrated density of about 65%, Sob1, Sob4 and Sob5 had about 70% and Sob3 showed the highest signal of all sobetiromes with about 80% (Fig.3.9B).

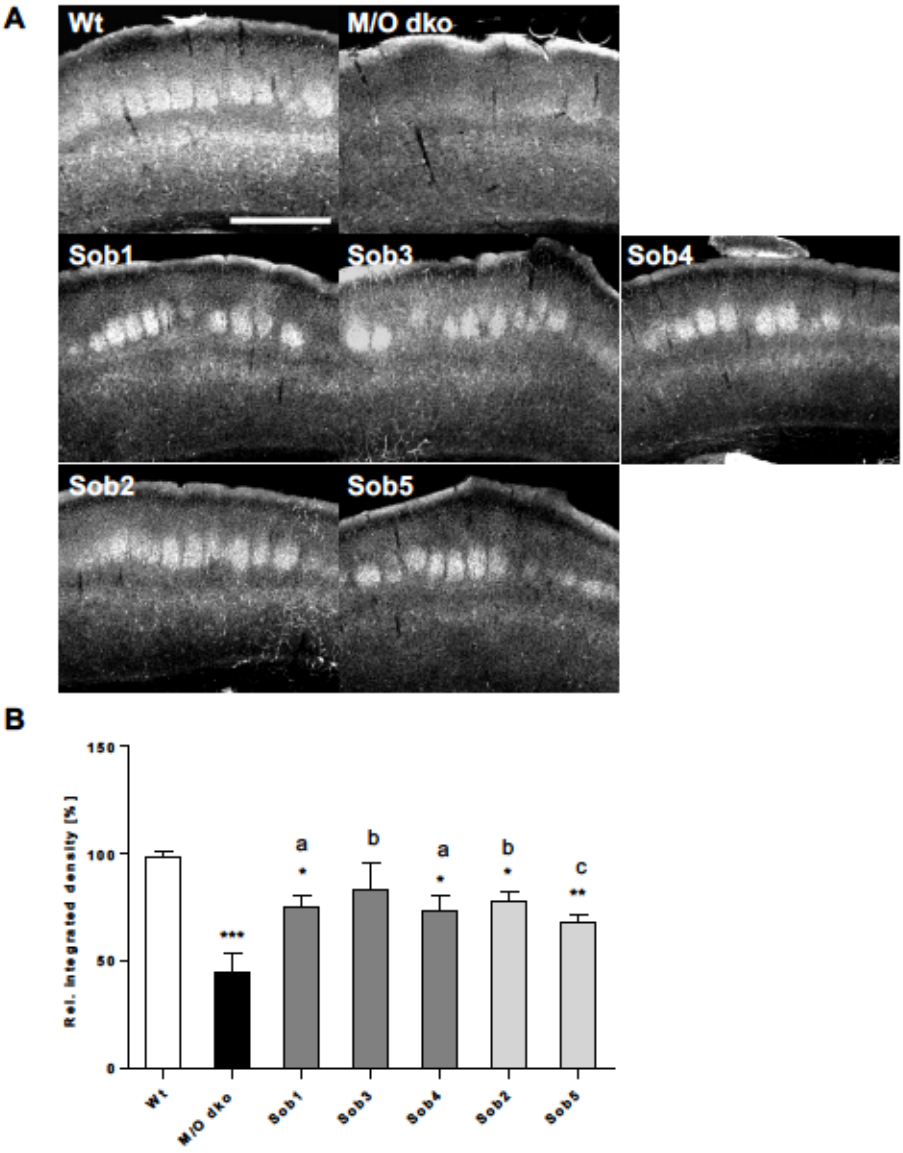


Fig.3.9 Effects of sobetiromes on GAD67 in the somatosensory cortex. At P12, GAD67 staining was present throughout all cortical layers with the strongest expression in the distinct barrels. M/O dko mice showed a significantly reduced GAD67 immunoreactivity. Different sobetirome compounds exhibited different efficacy in restoring this brain parameter ranging from 65% for Sob5 to 80% for the most efficient Sob3. Normalized to Wt: ***P<0.001, **P<0.01, *P< 0.05; normalized to M/O dko: a(**), b(***), c(*). n=3, Scale bar, 400 µm.

3.3.5 Effects of sobetiromes on myelination in the cerebral cortex

Another well-established action of TH during CNS development is the stimulation of myelination (Lee and Petratos, 2016) that in rodents, takes place primarily postnatally. In order to determine the effect of sobetiromes on myelination, coronal forebrain vibratome sections of P12 male mice were immunostained for Myelin Basic Protein (MBP), a crucial TH-regulated component of the myelin sheath (Rodríguez-Peña, 1999). Quantification of immunofluorescence stainings in the corpus callosum area (indicated by a red box in Fig.3.10) revealed a significant decrease in the expression level of MBP in the cerebral cortex of M/O dko animals (Fig.3.10A), with an almost 50% reduction in immunofluorescence intensity (Fig.3.10B). All sobetiromes were able to stimulate MBP expression with the exception of Sob4 that only partially normalized this parameter.

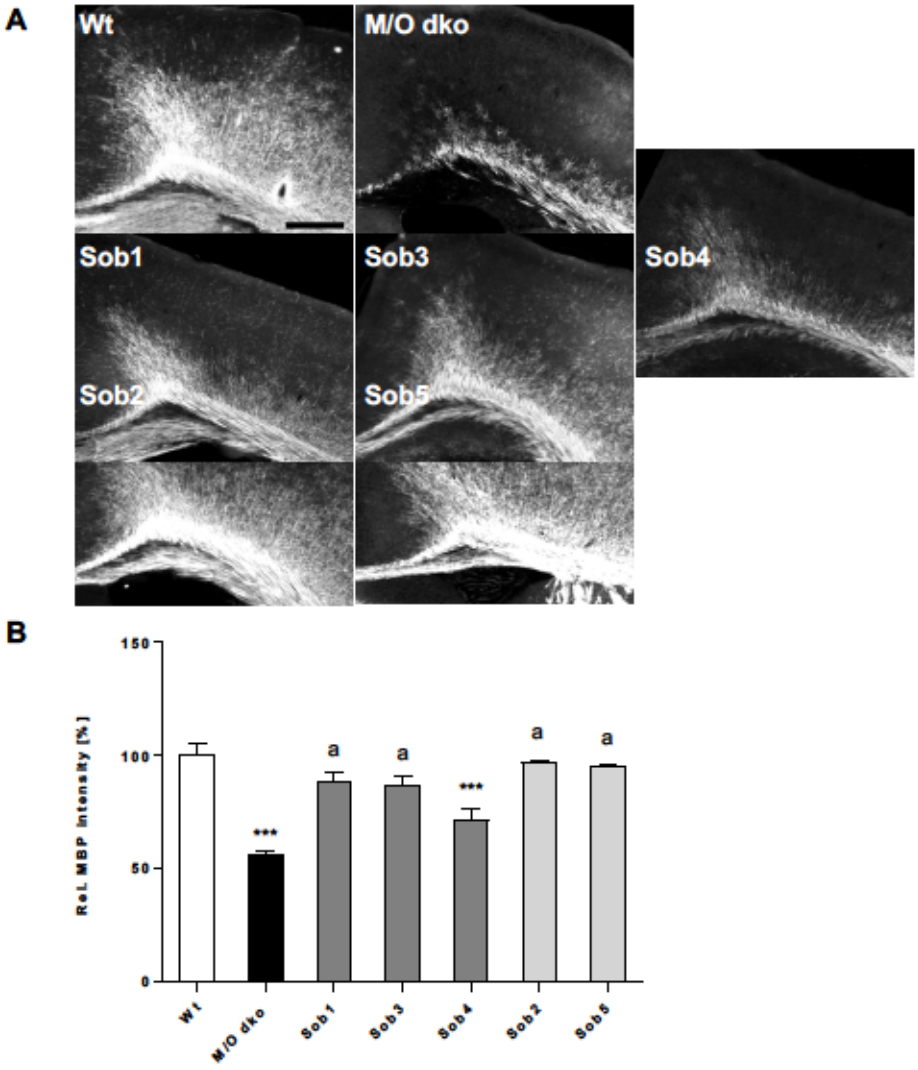


Fig.3.10 Effects of sobetiromes treatment on central myelination. Brain coronal sections derived from animals at the age of P12 were stained with anti-MBP antibody (A). Quantification of relative immunofluorescence intensities revealed an almost 50% reduction in the M/O dko mouse cortex compared to Wt. All sobetiromes except Sob4 showed similar MBP intensity values as Wt mice (B). Compared to Wt: ***P<0.001; compared to M/O dko: a(***). Red box represents the quantified area. n=3. Scale bar, 300 µm.

3.3.6 Effects of sobetiromes on cortical layering

M/O dko mice were reported to show an altered cortical layering with layer I and II being smaller (Mayerl et al., 2014).

To determine the effect of sobetiromes on the overall neuronal distribution, vibratome sections of P12 old male mice were immunostained against the neuronal marker NeuN (neuronal nuclei, Hexaribonucleotide Binding Protein-3). The thickness of cortical layers I-II could be determined due to distinct, layer specific NeuN staining patterns. M/O dko mice showed a significant reduction in the relative integrated density (Fig.3.11A, B) and a slight decrease in the thickness of the outer cortical layers I-II (Fig.3.11A, C), while the sobetirome treated animals showed similar NeuN immunoreactivity and layer thickness as the Wt animals.

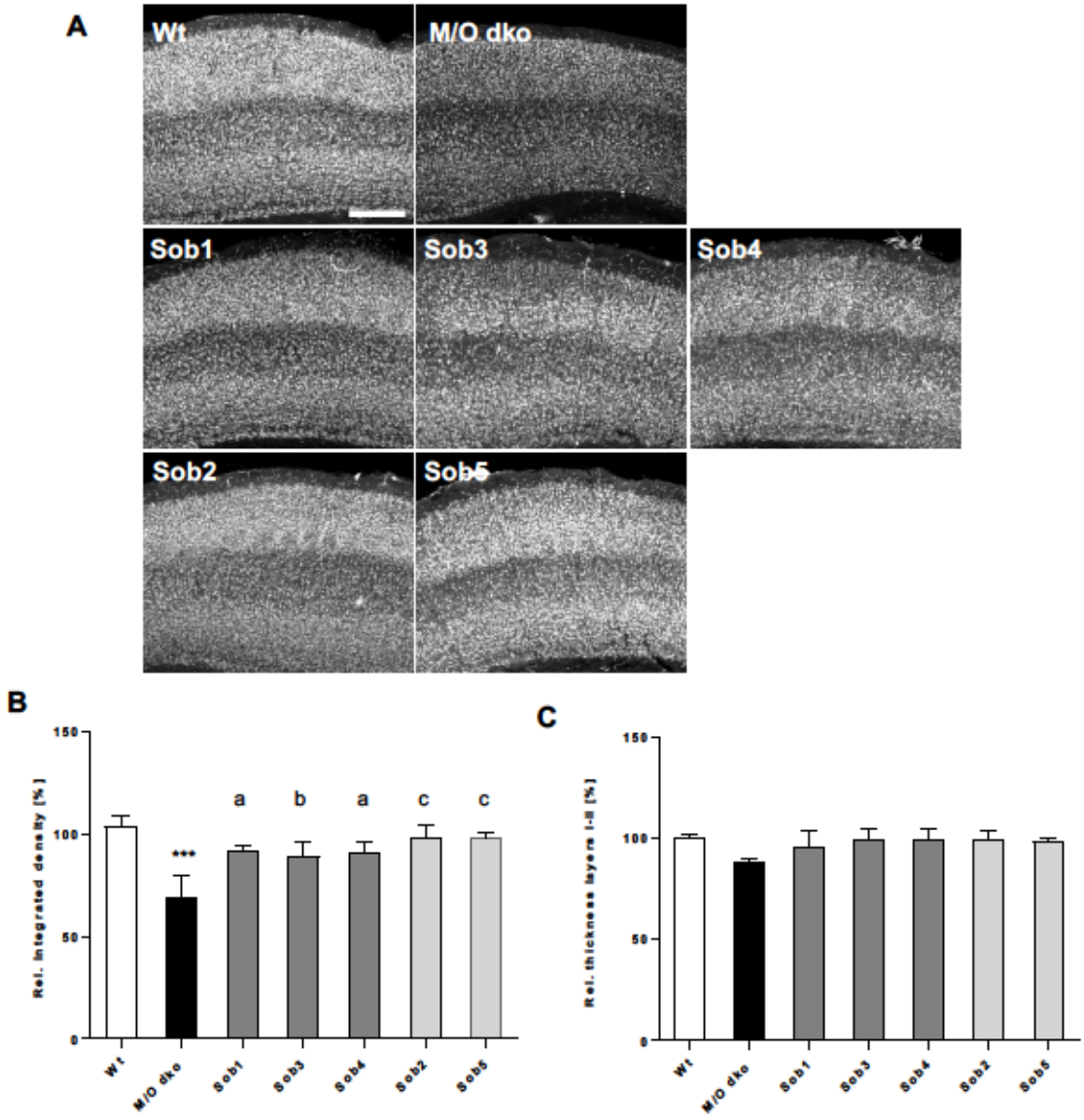


Fig.3.11 Effects of sobetiromes on cortical layering. Brain coronal sections derived from animals at the age of P12 were stained with anti-NeuN (A). Quantification of relative integrated density of the outer cortical layers I-II showed a significant reduction for M/O (B) while a slight decrease in M/O dko mice were obtained in the analysis of relative thickness of the layers I-II (C). Normalized to Wt: ***P<0.001; normalized to M/O dko: a(**), b(*), c(***). n=3; Scale bar, 300 µm.

4 Discussion

The critical role of TH for proper brain development has been underscored with the identification of patients carrying inactivating mutations in the TH transporter MCT8, leading to the Allan-Herndon-Dudley Syndrome (AHDS) (Friesema et al., 2004; Dumitrescu et al., 2004). At present, 320 patients with over 100 different mutations have been associated with this rare but severe form of psychomotor retardation composed of global developmental delay, intellectual disabilities and central hypotonia (Visser et al., 2016; Dumitrescu et al., 2004; Friesema et al., 2004). Their neurological symptoms together with the histopathophysiological findings suggest that the CNS of AHDS patients is in a general TH dependent state. Since circulating serum T3 levels are highly elevated it is reasonable to assume that the transport of T3 into the CNS across the BBB/BCSFB is dependent on MCT8. Indeed, postmortem data of MCT8-deficient patients have revealed anomalies compatible with brain hypothyroidism with alterations in myelination, neuronal differentiation and synaptogenesis already from prenatal stages (López-Espíndola et al., 2014). The central TH deprivation contrasts with the thyrotoxic situation in peripheral tissues of MCT8-deficient patients thereby making it challenging to find a suitable treatment strategy.

4.1 Animal models for studying MCT8 deficiency

In order to unravel the pathophysiological mechanism of MCT8 deficiency and to carry out preclinical studies to find a promising therapy approach, different animal models were used in the past years. The components involved in regulating local TH availability are evolutionary conserved and revealed a high homology between zebrafish, chicken, mouse and human.

Mct8 ko mice were the first animal model developed for studying AHDS (Trajkovic et al., 2007; Dumitrescu et al., 2006). Although these mice showed a thyroid hormone profile similar to that of the MCT8-deficient patients as well as a strongly reduced T3 uptake into the brain, they unexpectedly exhibited a rather mild phenotype without any neurological symptoms or behavioral impairments indicating the presence of compensatory mechanisms in these animals (Trajkovic et al., 2007; Dumitrescu et al., 2006; Wirth et al., 2009). This phenotypic discrepancy between Mct8-deficient mice and MCT8-deficient humans indicated the need for

the development of alternative models, particularly for unraveling the function of Mct8 in the CNS.

Zebrafish as a simple vertebrate model got increasingly popular to study neurological disorders due to its conserved organization of the CNS and thyroid system, including the HPT axis and the main genes involved in TH signaling (Bandmann and Burton, 2010; Vatine et al., 2013). Zebrafish mct8 was shown to be mainly expressed in the nervous system during embryonic and larval development, being strongly present in neurons and OL, and was further found to be highly expressed in the vascular system (Vatine et al., 2013). Induction of mct8-knockdown in zebrafish by morpholino-modified antisense oligonucleotides injection that either blocks the translation or leads to alternative splicing resulted in profound developmental and neurological disturbances. In particular, reduced brain size, especially of the cerebellum and mid-hindbrain, a curved body axis due to a deformed spinal cord, pericardial edema and reduced locomotor activity at 120 hours post fertilization were described (Campinho et al., 2014; Vatine et al., 2013). Strikingly, different expression patterns were observed for several T3-responsive genes including a decreased dio3 and increased dio1 expression as described by de Vrieze et al. (2014), whereas Vatine et al. (2013) observed no alteration in deiodinase expression. These differences might be related to off-target effects of morpholinos that can often lead to more severe phenotypes than those of the corresponding mutants (Zada et al., 2017).
Thereafter, mct8 ko zebrafish were generated using the Zinc Finger Nuclease technology that remarkably showed a much weaker phenotype than the knockdown zebrafish mutants. In particular, mct8 ko zebrafish larvae were all viable and fertile, the morphology of larvae and adult appeared normal and the expression of key TH-induced genes were not altered. Still, Mct8 ko zebrafish exhibit neurological and behavioral deficits. More precisely, they show an impaired number of mature myelinated axons, deficiency in the development of glial cells, axon branching and synaptic densities. The combined neural deficits alter behavioral performance, including locomotor activity, sleep and response to external stimuli (Zada et al., 2014).

Another model to study the function of MCT8 was the developing chicken. Mct8 expression was already visible at embryonic day 4 in CNS regions but was not found at the BBB during the entire period of embryonic development that contrasts to the mammalian and zebrafish situation (Bourgeois et al., 2016; Delbaere et al., 2016).
Mct8 knockdown by electroporation of Mct8-RNAi vector into the ventricular zone of the dorsolateral rhombencephalon at E3 allowed targeting cerebellar cells. PC precursors that

normally start expressing Mct8 soon after E3 (Delbaere et al., 2016) showed strongly reduced expression of differentiation marker and decreased expression of T3-responsive genes (Delbaere et al., 2017) upon Mct8 downregulation. Moreover, at E18, two days prior hatching, dendrites of Mct8-deficient PC was stunted and less complex. Likewise, GC precursors proliferation was reduced, and inward migration of post-mitotic GC was stalled (Delbaere et al., 2017). But it is still not clear whether Mct8 deficiency in chicken PC causes a persistent deficit in cell morphology and function, since in older Mct8/Oatp1c1 dko mice (P33 and P120) differences in the PC morphology were no longer observed (Mayerl et al., 2014).

Furthermore, the same method has been used to target neural progenitors of the chicken tectum which serves as a model for the mammalian cerebral cortex. Here, the differentiation of GABAergic neurons was strongly impaired upon Mct8 downregulation hinting for a severe dysfunction of the neural circuit (Vancamp et al., 2017). Moreover, reelin expression and the organization of radial glial cell fibers, two hallmarks of mammalian corticogenesis, were affected by Mct8 knockdown resulting in a disrupted signaling mechanism for neuronal migration.

So far, no knockout chicken model is available resembling pathophysiological processes of the human brain appropriately.

However, Mct8 expression as well as brain structure in zebrafish and chicken is very different from human and therefore, these two animal models might be mostly used as screening tools.

Since the mouse model for Mct8 deficiency showed a just mildly impaired T4 uptake and a compensatory increase in D2 activity in the brain another transporter, the Oatp1c1, was speculated to be involved in the TH supply of the mouse brain (van der Deurre et al., 2008; Pizzagalli et al., 2002; Sugiyama et al., 2003). Therefore, mice lacking both Mct8 and Oatp1c1 transporters have been generated which depict a hypothyroid situation in the CNS with clear defects in brain development and marked neurological abnormalities. In particular they show reduced myelination, delayed maturation of the cerebellum, an impaired neuronal differentiation of GABAergic neurons, different expression of TH-regulated genes as well as a hyperthyroid situation in the peripheral organs that fully reflect the patients´ phenotypes (Müller and Heuer, 2014, Mayerl et al., 2014). These features might result from reduced T3 and T4 transport across the brain barriers, or from the impaired uptake into target cells. In contrast to the single mutant animals that only resemble the peripheral TH status of the patients, M/O dko mice also show reduced TH content in the brain with consecutively severe neurological changes

(Mayerl et al., 2014). This makes the M/O dko mouse model to date the most suitable animal model for studying MCT8 deficiency and for testing novel therapeutic interventions.

Further, Mct8/Dio2 ko mice were proposed as an alternative and additional model for AHDS. These mice have a suppressed compensatory action of type 2 deiodinase (D2) which converts T4 into T3 in astrocytes leading to a 50% reduction in the cerebral T3 content, similar to what has been found in the cerebral cortex of the 30th gestational week fetus with mutations in MCT8. Moreover, they replicate the peripheral hyperthyroidism characteristic for MCT8-deficient patients (Liao et al., 2011; López-Espíndola et al., 2014). However, this model presents limitations as it is deprived of D2 whereas patients have an increased D2 activity in the brain. Additionally, these mice show no alterations in T4 content in the brain and therefore do not fully replicate the low T4 content in the brain of MCT8-deficient patients (López-Espíndola et al., 2014).

4.2 Therapeutic approaches in MCT8 deficiency

Although a variety of approaches have been tested, the therapeutic options for MCT8-deficient patients are still limited. The combination of hypothyroidism in the brain and hyperthyroidism in peripheral tissues presents an especially challenging problem. An optimal therapy for MCT8 deficiency would therefore be to i) safely alleviate the peripheral thyrotoxicosis and ii) to restore normal TH signaling in the brain.

A simple TH administration is not possible due to a blocked TH transport into the brain. Patients who have been treated with LT4 showed no improvement in the neurological function and even exhibited a further increase in serum T3 aggravating the thyrotoxic symptoms (Dumitrescu et al., 2004; Zung et al., 2011).

Administration of T4 in combination with PTU, an anti-thyroid drug, is aimed to normalize the serum concentration of TH and TSH. PTU does not only block the TH production but also inhibits D1 that reduces the conversion of T4 to T3 in peripheral tissues. This has been shown to normalize the symptoms of thyrotoxicosis as evidenced by improvement of body weight, heart rate and serum markers of tissue TH state (Visser et al., 2013; Wémeau et al., 2008). However, no change in the neurocognitive phenotype has been observed, since T4 still cannot cross the BBB in the absence of MCT8 (Visser et al., 2013; Wémeau et al., 2008; Groeneweg et al., 2017b). Moreover, long-term administration of PTU in hyperthyroid patients showed

severe side effects, such as agranulocytosis and liver failure (Vicente et al., 2017; Wu et al., 2017).

Since MCT8 deficiency is a monogenetic disorder, a gene therapy strategy seemed to be an attractive option to restore MCT8 function in tissues where it is of most physiological relevance. Recently, adeno-associated virus 9 (AAV9) vectors were tested to deliver human MCT8 (hMCT8) cDNA construct in the Mct8 ko mice. Thereby, the respective virus was injected intravenously (IV) or intracerebroventricularly (ICV) into postnatal day 1 Mct8 ko mice to achieve expression of hMCT8 and improve brain TH content. Compared to IV, ICV delivery of vector constructs resulted in more hMCT8 mRNA and protein in various brain regions. However, only IV delivered hMCT8 targeted the choroid plexus and slightly increased brain T3 content (Iwayama et al., 2016). A simple transfer of hMCT8 in brain cell membranes including neurons was obviously insufficient for elevating TH action inside the CNS, a finding that again underscores the relevance of functional Mct8 at brain barriers Therefore, a BBB-targeted viral transfer should be developed and tested to achieve functional MCT8 within the brain (Iwayama et al., 2016). In that regard, it would be relevant to study the gene delivery strategy in other models for human MCT8 deficiency, since the Mct8 ko mouse used in this study do not exhibit clear brain abnormalities.

The failure of LT4 treatment on neurodevelopment has been associated with an impaired transport of T4 across the BBB and into the target cells of brain. This hypothesis encouraged the development of another therapeutic approach for MCT8 deficiency, namely the use of TH analogs that ideally circumvent MCT8 for their cellular uptake, act like T3 inside the cell and are metabolized similarly to T3 to induce a natural TH signaling (Groeneweg et al., 2017b).
The synthetic Ditpa and the naturally occurring T3 metabolite Triac are examples for such compounds with strong thyromimetic actions. They have been tested in preclinical and clinical studies and have shown promising effects in the treatment of different diseases such as myxedema (Zondek and Leszynsky, 1956; Ibbertson and Fraser, 1959), goiter (Brenta et al., 2003) some cases of resistance to thyroid hormone (RTH) with TRβ mutations (Takeda et al., 1995), myocardial ischemia and hyperlipidemia (Oliver and Boyd, 1957).
Ditpa, the first tested TH analog for treating human MCT8 deficiency, was able to restore serum T3 and T4 levels in four AHDS patients that were initiated at the age of 8.5-25 months at a daily dose of 1-2 mg/kg for 26-40 months. These patients had almost normalized thyroid parameters in thyroid function tests, improved several markers of peripheral TH action and

showed a tendency of increasing weight, due to the reduction of serum T3. However, no changes in the neurocognitive phenotype were observed. This might be explained by the fact, that these patients received Ditpa treatment at the age of 8.5-25 months, a time point during which some neurological damages have already occurred and might be irreversible (Di Cosmo et al., 2009; Verge et al., 2012). Intriguingly, administration of Ditpa did not drastically decrease the TSH concentration but even increased the T4 level, suggesting that Ditpa might not be able to inhibit TSH secretion but rather reduces D1- and D2-mediated conversion of T4 to T3 in humans. Despite the peripheral improvements, direct evidence that Ditpa reduces the hypermetabolism of MCT8-deficient patients is lacking due to the difficulties in performing metabolic studies in affected individuals. Therefore, Mct8 ko mice were used to determine the effect of Ditpa on whole-body energy homeostasis and demonstrated to partially reverse the hypermetabolism characteristic of Mct8 deficiency (Ferrara et al., 2015). Importantly, Ditpa-treated mice demonstrated high prevalence of fatal cardiac arrhythmias during *in vivo* ischemia and reperfusion models. Therefore, caution must be taken for the clinical application in patients with risk for myocardial ischemia (Talukder et al., 2010).

Based on the same hypothesis, the potency of Triac to alleviate the thyrotoxic state in peripheral tissues has been intensively investigated. Triac was primarily recognized pharmacologically by its ability to reduce goiter size of hypothyroid rats (Burger et al., 1979). Later, it was shown in several studies that Triac is able to suppress the HPT axis, predominantly at the pituitary level by inhibiting serum TSH levels and hence reducing TH biosynthesis and normalizing thyrotoxic symptoms (Mirell et al., 1989; Liang et al., 1997; Juge-Aubry et al., 1995; Refetoff, 1993).
In humans, Triac application strongly reduced serum TSH levels in a dose-dependent manner and was therefore used as a therapy for thyroid carcinoma patients after total thyroidectomy (Müller-Gärtner and Schneider, 1988). Further, it was used for the treatment of RTH. This syndrome is caused by mutations in TRβ, which lead to a reduced response of target tissues to TH and increased serum TH concentrations most likely due to non-suppressed TSH levels.
In addition, *in vitro* and *in vivo* studies evaluating the action of Triac in Mct8 deficiency showed promising effects. In particular, Triac could improve PC development in culture and in mct8-deficient chicken and restore mRNA levels of myelin markers, such as myelin-basic protein (MBP) in mct8-deficient zebrafish model (Delbaere et al., 2017; Horn et al., 2013; Zada et al., 2016). Moreover, it was able to promote normal brain development in M/O dko mice if the treatment was started immediately after birth (Kersseboom et al., 2014).

Recently, Groeneweg et al., (2019) evaluated the effectiveness and safety of Triac treatment for peripheral thyrotoxicosis in pediatric and juvenile patients with MCT8 deficiency. Thereby, 45 patients required a mean dose of 38.3 µg/kg of bodyweight to attain T3 concentrations within the target range. After 12 months of treatment, patients showed a reduced serum T3 concentration as well as improvements in clinically relevant outcomes, including body weight, heart rate, blood pressure and biochemical markers of TH action in different tissues. Its effect on neurocognitive function in younger patients has to be still elucidated, since this trial did not include specific neurodevelopmental outcomes and enrolled patients of all ages. Therefore, most of these patients would have passed the small window of opportunity to modulate brain development. However, six of seven patients younger than 4 years of age gained head control and achieved independent sitting after 12 months of therapy (Groeneweg et al., 2019). Currently, another trial (NTC02396459) with focus on neurodevelopment was designed that will specifically enroll very young infants to attempt the earliest possible therapy start. It can be hypothesized that at the beginning of the treatment Triac has to be given in higher doses in order to completely suppress the HPT axis and to achieve a high concentration of Triac in the brain.

Altogether, these data indicate that multiple key features related to the peripheral phenotype of MCT8 deficiency can be improved under Triac treatment. Thereby, it is very likely that Triac reduces the serum T3 level through its strong inhibitory effect on pituitary TSH synthesis and secretion, thus normalizing the consequences of thyrotoxicosis in peripheral tissues. Although Triac has a potent TSH-suppressive effect, higher doses of Triac are required to achieve equal thyromimetic effects as T3, mainly due to its shorter half-life (6 h vs 23 h) (Kersseboom et al., 2014; Menegay et al., 1989). In turn, higher doses of Triac showed adverse side-effects, particularly inducing cardiac hypertrophy in rats (Lameloise et al., 2001).

In addition to Triac and Ditpa, other TH analogs have been considered to potentially improve treatment strategies in AHDS patients. Sobetirome, also known as GC-1 is a synthetic structural TH analog with tissue-specific TH actions due to its selective binding to TRβ over TRα. This makes it a useful chemical tool for studying the effects of thyroid hormone receptor (TR) isoform selectivity in cellular and animal models (Yoshihara et al., 2003; Scanlan, 2010; Placzek et al., 2016). Earlier studies suggested sobetirome as a novel cholesterol-lowering agent activating liver TRβ without overstimulating cardiac function by TRα activation in the heart (Trost et al., 2000). Further, it has been suggested that sobetirome can cross the BBB and

distribute to the CNS at levels that are sufficient to upregulate T3 target genes and to affect brain developmental pathways regulated by T3 (Trost et al., 2000; Manzano et al., 2007; Placzek et al., 2016). Thus, it was used for promoting myelin repair which is a proposed goal for preventing long-term neurodegeneration in multiple sclerosis (MS) and other demyelinating diseases such as X-linked adrenoleukodystrophy (X-ALD) (Hartley et al., 2019; Hartley et al., 2017). Based on the promising treatment results of sobetirome in demyelinating disorders and its ability to cross the BBB in murine models, a prodrug strategy has been recently established to further increase its brain specificity. Prodrugs of sobetirome have been developed with beneficial pharmacokinetic properties, including lower peripheral exposure, enhanced BBB penetration and increased CNS distribution of sobetirome by masking the carboxylic acid, a functional group known to impede BBB penetration (Bárez-López et al., 2018; Placzek et al., 2016; Hitchcock and Pennington, 2006).
Interestingly, the prodrugs are selectively converted to an active form of sobetirome by FAAH (Meinig et al., 2017; Hartely et al., 2019). The FAAH is highly expressed in the CNS with strongest immunoreactivity in pyramidal cells of the cerebral cortex and hippocampus as well as in PC of the cerebellar cortex (Tsou et al., 1998). In addition to the neuronal expression of FAAH, FAAH-immunoreactivity was also found in ependymal cells as well as in OL (Egertová et al., 2003).

As it would be expected for TRβ-selective T3 agonists, sobetirome and its prodrug Sob2 have shown to exert dose-dependent actions on the HPT axis. In euthyroid Wt mice treated once daily for 7 days, sobetirome and Sob2 suppressed circulating TSH. However, T4 was only moderately reduced after 7 days, suggesting a not yet established equilibrium within the HPT axis during this short dosing period. Extending the dosing duration from 7 to 29 days resulted in dose-dependent T4 and T3 depletion with fully depleted circulating T4 but normal TSH levels at a daily dose between 10 and 100 µg/kg/day. Despite the systemic T4 and T3 depletion, sobetirome- and Sob2-treated mice do not show signs of hypothyroidism (Ferrara et al., 2018). This might be explained by the presence and potency of these thyromimetics in the endogenous TH-depleted background.

Additionally, administration of sobetirome and its prodrugs to Mct8/Dio2-deficient mice, resulted in the depletion of circulating T4 and T3 and increased the expression of several T3-dependent genes in the brain (Bárez-López et al., 2018). Further, prodrug treatment in these

animals led to 1.8-fold higher sobetirome content in the brain and 2.5-fold less in plasma in comparison with the parent drug sobetirome (Bárez-López et al., 2018).

Taken together, sobetirome and its modified prodrugs have already been shown to have potent effects in different diseases and seem to be promising therapeutic candidates addressing the central hypothyroidism and the peripheral hyperthyroidism characteristic of MCT8 deficiency, though clinical and preclinical studies have not been performed yet. Therefore, in this study, M/O dko mice were used as an animal model in a unique approach to study and compare the impact and therapeutic potential of different sobetiromes on brain development regarding a treatment strategy for AHDS patients.

4.3 Central effects of sobetiromes during early development

4.3.1 Analysis of cortical thickness

TH exert important influences on cell maturation and migration in the cerebral cortex (Bernal et al., 2015). Here, TH are needed for proper arrangement of the six-layer-pattern, formed by the timely migration of cells originating in the ventricular neuroepithelium. It has been shown that hypothyroidism in developing rats decreases soma size of cortical neurons and density of axons which is why cells are packed closer together leading to less defined cortical layers (Eayrs and Goodhead, 1959; Berbel et al., 2001; Bernal et al., 2015). In addition, Berbel and colleagues described the irreversible effects of maternal and fetal hypothyroidism on the intrinsic organization and maturation of the neocortex (Berbel et al., 2001). Thereby, they could show that TH deprivation lead to cortical layering impairments in rats including blurred boundaries between the cortical layers and abnormal patches of high cell density in layer IV (Berbel et al., 2001). Further, in the barrel cortex that serves as a model for cortical development, congenital hypothyroidism resulted in a delay in barrel formation and reduction in barrel dimensions (Anderson, 2001).

Previous data have shown a reduced thickness of the cortical layers I-IV in the M/O dko mice (Mayerl et al., 2014).

In order to determine the effect of sobetiromes on the cortical thickness in the M/O dko mouse brain, immunohistochemical analysis of NeuN was performed in the somatosensory cortex to label all mature neurons. The relative integrated density was significantly reduced in the M/O dko mice whereas sobetirome treatment led to a normalization of neuron density as depicted in Fig.3.11B. In addition, the relative thickness of the layer I-II was slightly reduced in the M/O

dko mice as seen before while administration of all sobetiromes showed a recovery in the outer cortical layer thickness (Fig.3.11C).

It has been speculated that TH may influence neuronal migration through the regulation of the expression of the *Reln* gene. Reelin, the product of this gene, is an extracellular matrix protein produced by neurons that are located in layer I of the cerebral cortex and is an important factor for controlling cell positioning in the CNS (Marín-Padilla, 1990; Alvarez-Dolado et al., 1999; Bernal et al., 2015; Berbel et al., 2001). However, the exact underlying mechanism on cortical layering is not understood, yet.

4.3.2 Inhibitory circuits in the cerebral cortex

Impaired inhibitory circuits in the cerebral cortex are a hallmark of TH deficiency. GABAergic interneurons, the major inhibitory neurons in the CNS, release the neurotransmitter GABA in order to regulate the firing rate of target neurons. They are crucial in modulating cortical development, neuronal activity and plasticity in the developing brain and are also implicated in learning processes and locomotor activities (Rudy et al., 2011). Cortical GABAergic dysfunction has been linked to various psychiatric disorders, such as schizophrenia, bipolar disorder and autism (Chiapponi et al., 2016; Akbarian and Huang, 2006).

GABA is synthesized by two isoforms of the enzyme glutamic acid decarboxylase (GAD) GAD65 and GAD67. In most GABAergic interneurons, both GAD isoforms are co-expressed but differ in their subcellular localization as well as in their cellular abundance. GAD65 is predominantly localized to nerve terminals, whereas GAD67 is more widely distributed throughout the cell soma (Soghomonian and Martin, 1998; Erlander et al., 1991). Further, it has been shown, that GAD67 expression is sensitive to TH and that a hypothyroid situation in rats decreases the GAD67 expression in the cerebral cortex (Virgili et al., 1991). Moreover, reduced GAD67 expression has been reported to be linked to seizures that are also a typical clinical symptom of AHDS patients (Akbarian and Huang, 2006).

GAD67 was used in our study to determine the density of GABAergic-interneurons. In line with the previous findings that demonstrate a permanent reduction in the immunoreactivity of GAD67 in the somatosensory cortex of M/O dko mice (Mayerl et al., 2014), in this study we could show a normalization of the in GAD67 immunoreactivity mice with sobetirome treatments at a dose of 100 ng/g bw, but to a different extent (Fig.3.9). While Sob3 and Sob2 showed the best recovery in GAD67 expression Sob1, Sob4 and Sob5 seemed to be less efficient.

Depending on the brain region, GABAergic interneurons constitute 10%-25% of the total number of cortical neurons and are extremely diverse in their morphology and functional properties (Le Magueresse and Monyer, 2013). They have been classified into different subgroups that differ in morphology, intrinsic membrane properties, connectivity and are associated with the expression of specific calcium binding proteins, such as calretinin, calbindin and parvalbumin (Petilla Interneuron Nomenclature Group et al., 2008; Rudy et al., 2011). These proteins act as endogenous Ca^{2+} buffer modulating various Ca^{2+}-dependent processes in neurons such as regulation of action potential, promotion of neuronal bursting as well as protection of neurons against Ca^{2+} overload (Baimbridge et al., 1992; Jiang and Swann, 1997). In the absence of Ca^{2+}-binding proteins, there is an accumulation of Ca^{2+} excess inside the cytosol leading to hyperexcitability, which is supposed to cause neurodegeneration (Nag and Wadhwa, 1999).

The most common and well-studied GABAergic subgroup in the CNS is represented by PV+ cells, which account for ~40% of total GABAergic interneurons and are found in all layers of the cortex (Gibson et al., 1999; Xu et al., 2010). Immunocytochemical studies have demonstrated that PV+ interneurons belong to basket and chandelier cells, specialized interneurons that establish inhibitory synaptic contacts with initial axon segments of projection neurons, thereby controlling the output of the cerebral cortex. Further, developmental studies have shown that PV appears late in the development of the rodent cerebral cortex at around postnatal day 5 in the hippocampal region and at the beginning of the second postnatal week in the neocortex (del Río et al., 1994). Moreover, it was shown that in the brain of hypothyroid mice in a developmental stage, the number of PV+ interneurons decreases and this effect was normalized by exogenous T3 administration during early postnatal development (Uchida et al., 2014; Gilbert et al., 2007). In addition, postmortem studies of AHDS patients showed decreased PV immunoreactivity in the cerebral cortex and in the cerebellum, which might be one explanation for the locomotor deficits (López-Espíndola et al., 2014). A reduction in the number of PV+ cells was also observed in the M/O dko mouse model (Mayerl et al., 2014). In that study, the distribution of PV+ cells was assessed in the somatosensory and in the retrosplenial cortex by immunohistochemistry. The primary somatosensory cortex plays an important role in the innervation of the motor cortex to initiate and guide movements (Sreenivasan et al., 2016), whereas the retrosplenial cortex is mainly involved in cognitive function, such as spatial memory and learning. Dysfunction of the latter brain area might contribute to memory disorders and spatial disorientation (Vann et al., 2009). According to these findings, PV+ interneurons were evaluated in the somatosensory and retrosplenial cortex.

In agreement with the previous data, our *in vivo* studies showed a decrease in PV+ cells under TH deprivation. *In vivo,* all sobetiromes were able to normalize the number of PV-expressing cells in the cerebellum (Fig.3.6), while treatment with Sob2, Sob4 and Sob5 showed the best immunoreactivity in the retrosplenial as well as in the somatosensory cortex *in vivo* (Fig.3.7).

Apart from PV-expressing neurons, calretinin-immunoreactive neurons were quantified as well. These neurons form networks of cells that are electronically coupled. They control the activity of other subpopulations of GABA-containing inhibitory interneurons, which are known to be important for motor control (Jiang and Swann, 1997).
In our study, calretinin expression in the somatosensory cortex of M/O dko mice seemed to be elevated in cortical layer II/III, while treatment with all sobetiromes showed a similar molecular thickness as the Wt animals, showing no difference in calretinin-positive cell number in layer II/III (Fig.3.8).

Taken together, all sobetiromes were able to exert thyromimetic action in the cerebral cortex, but with a remarkable different efficacy. This might be explained by the usage of different transporters for cellular drug uptake of the different sobetiromes, with alterations in transporter distribution and activity patterns in PV-, calretinin- and/or GAD67-expressing cells. So far, the exact transport mechanisms for sobetiromes have not been unraveled yet.

4.3.3 Promotion of cerebellar differentiation

Another striking hallmark of human MCT8 deficiency is a severe impairment of locomotor control including spasticity, poor head control and the inability to stand or walk (Schwartz et al., 2005; Visser et al., 2011). Amongst other CNS regions, the cerebellum is involved in coordination and fine motor control and hence permanent cerebellar defects are thought to determine clinical deficits in MCT8-deficient patients.
A number of studies have shown that TH play a crucial role in cerebellar development and that TH deficiency results in abnormal cerebellar growth and impairs neural differentiation causing irreversible neurological alterations (Vincent et al., 1982; Pasquini and Adamo, 1994; Koibuchi, 2008). In rodents, TH affects development of basically all cerebellar cell types during the first two weeks of postnatal life. Especially the migration of GC, the expression of PV+ interneurons as well as the differentiation of PC, the key player in maintaining normal motor function, are dependent on the presence of sufficient TH (Bernal, 2005). Under hypothyroid

conditions, all these processes seem to be retarded leading to severe neurological symptoms such as ataxia and poor fine motor movement (Anderson et al., 2003).

These observations emphasize the importance of proper TH supply during cerebellar development. Effects of TH on PC dendritogenesis can be assessed reliably in *in vitro* studies using a mixed cerebellar cell culture system under defined serum-free conditions (Heuer and Mason, 2003). Absence of TH in Wt cultures results, as expected, in a strongly retarded development of the PC dendritic tree while adding a physiological concentration of 1 nM T3 to the culture medium causes a threefold increase in dendritic outgrowth of PC during a 14 day culture period (Kimura-Kuroda et al., 2002; Heuer and Mason, 2003).

Based on the advantages of this *in vitro* system, the first approach of the present study was to analyze and to compare the thyromimetic effects of sobetiromes (Sob1-Sob5) on PC development and on PV+ interneurons. All sobetiromes could normalize the dendritic outgrowth and restore the number of PV+ cells, even though with different efficacy. For Sob2 and Sob5, only a dose of 0.1 nM was needed to normalize both parameters, whereas higher doses were needed for Sob1 and Sob3 (10 nM) as well as for Sob4 (1 nM) to exert similar effects. These results point to a better transport of Sob2 and Sob5 into the neurons, probably due to a higher transporter expression at the BBB or due to additional transport possibilities for these two Sobs (Fig.3.1; Fig.3.2). Further, our data suggest that the pharmacological dosage used for the sobetiromes was sufficient to stimulate TRα induced PC dendritogenesis. This was surprising, since it was shown that sobetiromes have an approximately 10-fold higher selectivity for the TRβ isoform over TRα (Scanlan, 2010).

Moreover, Meinig and colleagues suggested that sobetirome prodrugs have a better ability then sobetirome to enter cells in which they are catalyzed by the FAAH to the active compound (Meinig et al., 2017). Indeed, we could demonstrate that the two prodrugs Sob2 and Sob5 are cleaved by the FAAH into their active compound, since their induced PC outgrowth as well as the number of PV+ cells were significantly reduced by using two different inhibitors (PF-3845, URB937) of FAAH (Fig.3.3; Fig.3.4).

In line with these *in vitro* data, our in *vivo studies* in M/O dko mice demonstrate that treatment between P1 and P12 with Sob1-Sob5 (100 ng/g bw) stimulates cerebellar PC dendritogenesis and branching and results in a complete normalization of the molecular layer thickness (Fig.3.5). This confirms the strong thyromimetic effect of the sobetiromes on cerebellar development and PC dendritogenesis.

4.3.4 Analysis of myelination

A further well-established TH-regulated process in brain development is myelination. This process is crucial for a rapid and saltatory propagation of axon potentials and neuronal connectivity and is consequently mandatory for normal motor, sensory and higher cognitive function (Ahlgren et al., 1997). TH regulate the morphological and functional maturation of OL by stimulating the expression of prominent myelin markers such as proteolipid protein (PLP), myelin-associated glycoprotein (MAG) and myelin basic protein (MBP). Thereby, MBP expression is directly regulated by T3 via a TRE in the promoter region and becomes responsive especially in the first postnatal week (Bernal, 2005; Farsetti et al., 1992; Strait et al., 1997). Additionally, TH trigger the cell-cycle exit of OPC to become mature, contact multiple axons and start forming myelin sheaths. In mice, OPC proliferation and differentiation peaks at P14 and continues throughout the first months, while in humans the main phase of OL development starts in the third trimester and continues until 5 years of age (Anderson et al., 2003; Rodríguez-Peña et al., 1993; Barradas et al., 2001).

Hypomyelination is frequently observed in young MCT8-deficient patients by MRI and has been confirmed by analyzing postmortem brain samples. It is still a matter of debate, whether this phenotype is caused by a comprised MCT8-mediated T3 uptake into OL and OPC or a general TH-deficient state in the MCT8-deficient brain. Recent *in vitro* studies showed that human embryonic stem cell-derived OL express MCT8. Further, pharmacological and genetic blockade of MCT8 induced significant OL apoptosis resulting in impaired myelination. These results suggest a potential role of MCT8 in oligodendroglia cells and point to cell-intrinsic defect on OL differentiation and myelin gene expression (Lee et al., 2017; Billon et al., 2002).

In the current study, myelination was analyzed in M/O dko mice treated with sobetiromes postnatally (P1-P12). In agreement with previous findings of FAAH expression in OL (Egertová et al., 2003) the current study could show that sobetiromes can enter OL in the absence of Mct8 and Oatp1c1 and that the most pronounced effects on myelination were achieved with the prodrugs Sob2 and Sob5 (100 ng/g bw) whereas Sob1 and Sob3 showed a mild and Sob4 a weak improvement of myelination (Fig.3.10).

It is known, that TRα aswell as TRß are required for a proper and coordinated development of myelination. Hence, our data strongly suggest sobetiromes to promote OPC differentiation and lead to a fully recovered myelin content in the murine M/O dko brain by stimulation of both, TRα and TRß despites their higher selectivity for TRß. Best results for myelination were shown after treatment with Sob2 and Sob5, whereas Sob4 induces weaker myelination. Therefore, especially Sob2 and Sob5 are promising candidates to replace T3 in regulating myelination

during early development of the CNS improving the nerve conduction velocity and leading to a better innervation of the musculature in AHDS patients.

4.4 Conclusions and future perspectives

In vitro studies of mixed cerebellar cultures as well as *in vivo* studies in Mct8/Oatp1c1 dko mice were performed to investigate novel treatment perspectives with new TH analogs for patients with AHDS. The two prodrugs Sob2 and Sob5 provided the best results in all conducted experiments, representing the most appropriate candidates for further pharmacological testing. *In vitro*, they showed the same effects on PC development and on the generation of PV+ cells like 1 nM T3 with a concentration of just 0.1 nM and thus 10x less than T3. Also, in our *in vivo* experiments Sob2 and Sob5 exerted the strongest thyromimetic effects in the developing mouse brain on almost all analyzed processes. At a daily dose of 100 ng/g bw for the first 12 postnatal days, both prodrugs were able to correct central abnormalities such as reduced myelination, delayed cerebellar development and impairments in the development of the cortical GABAergic system. The same applies to Sob3, though *in vitro* a 10-fold higher concentration was necessary for similar effects in the PC outgrowth compared to Sob2 and Sob5. Interestingly, Sob1 and Sob4 could also normalize these brain parameters to some extent but they showed cell-specific differences. As an example, Sob1 was less effective in normalizing PV-expression, while Sob4 was less effective in normalizing myelination in the cerebral cortex. This indicates a reduced entry of Sob1 and Sob4 into the M/O dko brain or into specific cell types.

However, further investigations have to be considered to unravel the complex interactions and pharmacological effects and to understand the exact mechanisms of transport and action for sobetiromes, in particular in brain cells. It remains largely illusive how sobetiromes enter their target cells and whether these processes differ between species. CNS uptake differences may lead to zonal exposure differences within the brain and might explain the variations in the treatment outcome of sobetiromes. Another explanation could be a difference in TH sensitivity of different cell types and tissues.

Furthermore, it has to be elucidated at which time point the BBB gets mature and from when TH transport is only based on Mct8 and Oatp1c1 to address the correct timing of TH analog treatment onset. So far, it is not known yet at what developmental phase these analogs should be applied to have a reasonable chance to modulate brain development. It was shown that MCT8 is already highly expressed in some cell types during early fetal brain development, that at that

stage depend on sufficient TH concentrations (Bernal, 2011; Bernal et al., 2015). The presence of neuropathological changes in postmortem studies of a MCT8-deficient fetus showed a delay in cortical and cerebellar development and myelination, loss of PV-expression, an impaired axonal maturation as well as diminished PC differentiation (López-Espíndola et al., 2014), suggesting that MCT8 may indeed be critical in some cells during early developmental stages.

From a clinical point of view, a rapid diagnosis and thus an early treatment start seems to be crucial for the patients´ wellbeing. Hence, it should be considered to include T3 besides TSH and T4 to the neonatal screening to not just identify newborns with congenital hypothyroidism but also with MCT8 mutations. The failure of Ditpa to rescue the neurological phenotype of MCT8-deficient patients has been attributed to the late initiation of treatment (Ferrara et al., 2014; Verge et al., 2012). Therefore, future research should be dedicated to guarantee early detection of disease, since an early, maybe even prenatal treatment of the pregnant MCT8 carrier mother might be able to prevent the brain damage caused by TH abnormalities during embryonic life. Ditpa and Triac have already been shown to cross the placenta in mice and humans (Ferrara et al., 2014; Asteria et al., 1999; Cortelazzi et al., 1999). It would be interesting to evaluate if sobetiromes are able to pass the placenta as well. If so, the opportunity of prenatal treatment of AHDS positive unborn children via systemic application of sobetiromes to the mother would be given. Of note, medical and ethical considerations would have to be targeted prior, since the procedure used for prenatal testing require a sample of amniotic fluid sample which carry the risk of injuring the fetus or losing the pregnancy.

On the other hand, most of the human AHDS patients are diagnosed at later timepoints in life. Taking into consideration, that in the present study mice have been treated with TH analogs until P12, a developmental stage comparable to a human brain around birth (Anderson et al., 2003), it is necessary to perform sobetirome treatment in older M/O dko animals in order to further evaluate the therapeutic potential of sobetiromes in normalizing brain parameters at later developmental stages and to assess the putative long-term beneficial effects of these drugs. Moreover, previous studies on adult M/O dko mice revealed drastic locomotor impairments such as limited motor coordination and reduced muscle strength. In light of these findings, it will be necessary to determine if sobetirome treatment during the first postnatal weeks exerts any improvement in the motor control later in life. Therefore, behavioral tests like accelerating Rotarod in adult M/O dko mice have to be performed after treatment.

Taken together, 75 years after the initial description of AHDS TH analog treatment strategy seems so far the most promising approach. Sobetiromes have the potential to address not just the peripheral hyperthyroidism but also the cerebral hypothyroidism, which is characteristic for MCT8-deficient patients. Additionally, sobetiromes provide an increased CNS specificity compared to other TH analogs, thereby showing advantageous pharmacokinetic effects. In this study, we could demonstrate that sobetiromes and their prodrugs were able to cross the BBB independently of Mct8, to replace TH in the CNS and to overcome developmental consequences of central TH deficiency by normalizing different brain parameters in the M/O dko mouse model. Especially the FAAH-activated prodrugs Sob2 and Sob5 increase the distribution of the active drug in the CNS. Therefore, they can be expected to confer better therapeutic benefits than Triac, providing an increased pharmacologic specificity and efficacy and in addition avoiding peripheral thyrotoxic adverse effects.

5 References

Abel, E.D., Ahima, R.S., Boers, M.E., Elmquist, J.K., and Wondisford, F.E. (2001). Critical role for thyroid hormone receptor beta2 in the regulation of paraventricular thyrotropin-releasing hormone neurons. J. Clin. Invest. 107, 1017–1023.

Abbott, N.J, Rönnbäck, L., and Hansson, E. (2006). Astrocyte-endothelial interactions at the blood-brain barrier. Nat. Rev. Neurosci. 1, 41-53.

Ahlgren, S.C., Wallace, H., Bishop, J., Neophytou, C., and Raff, M.C. (1997). Effects of thyroid hormone on embryonic oligodendrocyte precursor cell development in vivo and in vitro. Mol. Cell. Neurosci. 9, 420–432.

Aird, W.C. (2007). Phenotypic heterogeneity of the endothelium: Structure, function and mechanisms. Circ. Res. 100, 158-173.

Akanuma, S., Hirose, S., Tachikawa, M., and Hosoya, K. (2013). Localization of organic anion transporting polypeptide (Oatp) 1a4 and Oatp1c1 at the rat blood-retinal barrier. Fluids Barriers CNS. 10, 29.

Akbarian, S., and Huang, H.-S. (2006). Molecular and cellular mechanisms of altered GAD1/GAD67 expression in schizophrenia and related disorders. Brain Res. Rev. 52, 293–304.

Alkemade, A., Friesema, E.C., Unmehopa, U.A., Fabriek, B.O., Kuiper, G.G., Leonard, J.L., Wiersinga, W.M., Swaab, D.F., Visser, T.J., and Fliers, E. (2005). Neuroanatomical pathways for thyroid hormone feedback in the human hypothalamus. J. Clin. Endocrinol. Metab. 90, 4322–4334.

Alkemade, A., Friesema, E.C., Kalsbeek, A., Swaab, D.F., Visser, T.J., and Fliers, E. (2011). Expression of thyroid hormone transporters in the human hypothalamus. J. Clin. Endocrinol. Metab. 96, 967-971.

Allan, W., Herndon, C.N., and Dudley, F.C. (1944). Some examples of the inheritance of mental deficiency: apparantly sex-linked idiocy and microcephaly. Am. J. Ment. Defic. 48, 325-334.

Alvarez-Buylla, A., and Garcia-Verdugo, J.M. (2002). Neurogenesis in adult subventricular zone. J. Neurosci. 22, 629-634.

Alvarez-Dolado, M., González-Moreno, M., Valencia, A., Zenke, M., Bernal, J., and Muñoz, A. (1999). Identification of a mammalian homologue of the fungal Tom70 mitochondrial precursor protein import receptor as a thyroid hormone-regulated gene in specific brain regions. J. Neurochem. 73, 2240–2249.

Andersen, P.N., Skogli, E.W., Hovik, K.T., Geurts, H., Egeland, J., and Oie, M. (2015). Working memory arrest in children with high-functioning autism compared to children with

attention-deficit/hyperactivity disorder: results from a 2-year longitudinal study. Autism. 19, 443-450.

Anderson, G.W. (2001). Thyroid hormones and the brain. Front. Neuroendocrinol. 22, 1–17.

Anderson, G.W., Schoonover, C.M., and Jones, S.A. (2003). Control of thyroid hormone action in the developing rat brain. Thyroid. 13, 1039–1056.

Anzai, R., Adachi, M., Sho, N., Muroya, K., Asakura, Y., and Onigata, K. (2012). Long-term 3,5,3′-triiodothyroacetic acid therapy in a child with hyperthyroidism caused by thyroid hormone resistance: pharmacological study and therapeutic recommendations. Thyroid. 22, 1069-1075.

Aschner, M., Costa, L.G. (2015). Environmental Factors in Neurodevelopmental and Neurodegenerative Disorders (Elsevier Inc.).

Asteria, C., Rajanayagam, O., Collingwood, T.N., Persani, L., Romoli, R., Mannavola, D., Zamperini, P., Buzi, F., Ciralli, F., Chatterjee, V.K., et al. (1999). Prenatal diagnosis of thyroid hormone resistance. J. Clin. Endocrinol. Metab. 84, 405–410.

Baimbridge, K.G., Celio, M.R., and Rogers, J.H. (1992). Calcium-binding proteins in the nervous system. Trends Neurosci. 15, 303–308.

Bandmann, O., and Burton, E.A. (2010). Genetic zebrafish models of neurodegenerative diseases. Neurobiol. Dis. 40, 58-65.

Baptista, C.A., Hatten, M.E., Blazeski, R., and Mason, C.A. (1994). Cell-cell interactions influence survival and differentiation of purified Purkinje cells in vitro. Neuron. 12, 243–260.

Barca-Mayo, O., Liao, X.-H., Alonso, M., Di Cosmo, C., Hernandez, A., Refetoff, S., and Weiss, R.E. (2011). Thyroid hormone receptor α and regulation of type 3 deiodinase. Mol. Endocrinol. 25, 575–583.

Bárez-López, S., Hartley, M.D., Grijota-Martínez, C., Scanlan, T.S., and Guadaño-Ferraz, A. (2018). Sobetirome and its Amide Prodrug Sob-AM2 Exert Thyromimetic Actions in Mct8-Deficient Brain. Thyroid. 28, 1211–1220.

Barradas, P.C., Vieira, R.S., and De Freitas, M.S. (2001). Selective effect of hypothyroidism on expression of myelin markers during development. J. Neurosci. Res. 66, 254-261.

Baxi, E.G., Schott, J.T., Faichild, A.N., Kirby, L.A., Karani, R., Uapinyoying, P., Pardo-Villamizar, C., Rothstein, J.R., Bergles, D.E., and Calabresi, P.A. (2014). A selective thyroid hormone ß receptor agonist enhances human and rodent oligodendrocyte differentiation. Glia. 62, 1513-1529.

Baxter, J.D., Dillmann, W.H., West, B.L., Huber, R., Furlow, J.D., Fletterick, R.J., Webb, P., Apriletti, J.W., and Scanlan, T.S. (2001). Selective modulation of thyroid hormone receptor action. J. Steroid Biochem. Mol. Biol. 76, 31-42.

Baxter, J.D., and Webb, P. (2009). Thyroid hormone mimetics: potential applications in

atherosclerosis, obesity and type 2 diabetes. Net. Rev. Drug Discov. 8, 308-320.

Becker, H.M., Mohebbi, N., Perna, A., Ganapathy, V., Capasso, G., and Wagner, C.A. (2010). Localization of members of MCT monocarboxylate transporter family Slc16 in the kidney and regulation during metabolic acidosis. Am. J. Physiol. Renal Physiol. 299, 141-154.

Berbel, P., Ausó, E., García-Velasco, J.V., Molina, M.L., and Camacho, M. (2001). Role of thyroid hormones in the maturation and organisation of rat barrel cortex. Neuroscience. 107, 383–394.

Berbel, P. Guadaño-Ferraz, A., Angulo, A., and Ramòn Cerezo, J. (1994). Role of thyroid hormones in the maturation of interhemispheric connections in rats. Behav. Brain Res. 81, 293-308.

Bernal, J. (2000). Thyroid Hormones in Brain Development and Function. In Endotext, K.R. Feingold, B. Anawalt, A. Boyce, G. Chrousos, K. Dungan, A. Grossman, J.M. Hershman, G. Kaltsas, C. Koch, P. Kopp, et al., eds. (South Dartmouth (MA): MDText.com, Inc.).

Bernal, J. (2002). Action of thyroid hormone in brain. J. Endocrinol. Invest. 25, 268-288.

Bernal, J. (2005). Pathophysiology of thyroid hormone deficiency during fetal development. J. Pediatr. Endocrinol. Metab. 18, 1253–1256.

Bernal, J. (2007). Thyroid hormone receptors in brain development and function. Nat. Clin. Pract. Endocrinol. Metab. 3, 249-259.

Bernal, J. (2011). Thyroid hormone transport in developing brain. Curr. Opin. Endocrinol. Diabetes Obes. 18, 295–299.

Bernal, J., Guadaño-Ferraz, A., and Morte, B. (2015). Thyroid hormone transporters-functions and clinical implications. Nat. Rev. Endocrinol. 11, 690.

Bianco, A.C., and Kim, B.W. (2006). Deiodinases: implications of the local control of thyroid hormone action. J. Clin. Invest. 116, 2571–2579.

Bianco, A.C., Salvatore, D., Gereben, B., Berry, M.J., and Larsen, P.R. (2002). Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr. Rev. 23, 38–89.

Biebermann, H., Ambrugger, P., Tarnow, P., von Moers, A., Schweizer, U., and Grueters, A. (2005). Extended clinical phenotype, endocrine investigations and functional studies of a loss-of-function mutation A150V in the thyroid hormone specific transporter MCT8. Eur. J. Endocrinol. 153, 359-366.

Billon, N., Jolicoeur, C., Tokumoto, Y., Vennström, B., and Raff, M. (2002). Normal timing of oligodendrocyte development depends on thyroid hormone receptor alpha 1 (TRα1). EMBO J. 21, 6452–6460.

Blank, B., Pfeiffer, F.R., Greenberg, C.M., and Kerwin, J.F. (1963). Thyromimetics. II. The Synthesis and Hypocholesteremic Activity of some Beta-Diethylaminoethyl Esters of Iodinated

Thyroalkanoic Acids. J. Med. Chem. 6, 560-563.

Bleicher, L., Aparicio, R., Nunes, F.M., Martinez, L., Gomes Dias, S.M., Figueira, A.C., Santos, M.A., Venturelli, W.H., et al. (2008). Structural basis of GC-1 selectivity for thyroid hormone receptor isoforms. BMC Struct. Biol. 8, 8.

Bochukova, E., Schoenmakers, N., Agostini, M., Schoenmakers, E., Rajanayagam, O., Keogh, J.M., Henning, E., Reinemund, J., Gevers, E., Sarri, M., Downes, K., Offiah, A., et al. (2012). A mutation in the thyroid hormone receptor alpha gene. N. Engl. J. Med. 367, 1474.

Boger, D.L., Fecik, R.A., Patterson, J.E., Miyauchi, H., Patricelli, M.P., and Cravatt, B.F. (2000). Fatty acid amide hydrolase substrate specificity. Bioorg. Med. Chem. Lett. 10, 2613-1616.

Boukhtouche, F., Brugg, B., Wehrlé, R., Bois-Joyeux, B., Danan, J.-L., Dusart, I., and Mariani, J. (2010). Induction of early Purkinje cell dendritic differentiation by thyroid hormone requires RORα. Neural. Dev. 5, 18.

Bourgeois, N.M., Van Herck, S.L., Vancamp, P., Delbaere, J., Zevenbergen, C., Kersseboom, S., Darras, V.M., and Visser, T.J. (2016). Characterization of Chicken Thyroid Hormone Transporters. Endocrinology. 157, 2560-2574.

Bradley, D.J., Towle, H.C., and Young, W.S. (1992). Spatial and temporal expression of alpha- and beta-thyroid hormone receptor mRNAs, including the beta 2-subtype, in the developing mammalian nervous system. J. Neurosci. 12, 2288–2302.

Braun, D., Kinne, A., Bräuer, A.U., Sapin, R., klein, M.O., Köhrle, J., Wirth, E.K., and Schweizer, U. (2011). Developmental and cell type-specific expression of thyroid hormone transporters in the mouse brain and in primary brain cells. Glia. 59, 463-471.

Braun, D., and Schweizer, U. (2018). Thyroid Hormone Transport and Transporters. Vitam. Horm. 106, 19–44.

Brent, G.A., Moore, D.D., Larsen, P.R. (1991). Thyroid hormone regulation of gene expression. Annu. Rev. Physiol. 53, 17-35.

Brent, G.A. (2012). Mechanisms of thyroid hormone action. J. Clin. Invest. 122, 3035–3043.

Brenta, G., Schnitman, M., Fretes, O., Facco, E., Gurfinkel, M., Damilano, S., Pacenza, N., Blanco, A., Gonzalez, E., and Pisarev, M.A. (2003). Comparative efficacy and side effects of the treatment of euthyroid goiter with levo-thyroxine or triiodothyroacetic acid. J. Clin. Endocrinol. Metab. 88, 5287–5292.

Brightman, M.W., and Reese, T.S. (1969). Junctions between intimately apposed cell membranes in the vertebrate brain. J. Cell Biol. 40, 648-677.

Burger, A.G., Engler, D., Sakoloff, C., and Staeheli, V. (1979). The effects of tetraiodothyroacetic and triiodothyroacetic acids on thyroid function in euthyroid and hyperthyroid subjects. Acta Endocrinol. 92, 455-467.

Burmeister, L.A., Pachucki, J., Germain, D.L. (1997). Thyroid hormones inhibit type 2 iodothyronine deiodinase in the rat cerebral cortex by both pre- and posttranslational mechanisms. Endocrinology. 138, 5231-5237.

Calzà, L., Baldassarro, V.A., Fernandez, M., Giuliani, A., Lorenzini, L., and Giardino, L. (2018). Thyroid Hormone and the White Matter of the Central Nervous System: From Development to Repair. Vitam. Horm. 106, 253–281.

Campinho, M.A., Saraia, J., Florindo, C., and Power, D.M. (2014). Maternal thyroid hormones are essential for neural development in zebrafish. Mol. Endocrinol. 28, 1136-1149.

Ceballos, A., Belinchon, M.M., Sanchez-Mendoza, E., Grijota-Martinez, C., Dumitrescu, A.M., Refetoff, S., Morte, B., and Bernal, J. (2009). Importance of monocarboxylate transporter 8 for the blood-brain barrier-dependent availability of 3,5,3′-triiodo-L-thyronine. Endocrinology. 150, 2491-2496.

Celio, M.R. (1986). Parvalbumin in most gamma-aminobutyric acid-containing neurons of the rat cerebral cortex. Science. 28, 995-997.

Chan, S.Y., Martin-Santos, A., Loubiere, L.S., Gonzalez, A.M., Stieger, B., Logan, A., McCabe, C.J., Franklyn, J.A., and Kilby, M.D. (2011). The expression of thyroid hormone transporters in the human fetal cerebral cortex during early development and in N-Tera-2 neurodifferentiation. J. Physiol. 589, 2827-2845.

Chanoine, J.P., Braverman, L.E., Farwell, A.P., Safran, M., Alex, S., Dubord, S., and Leonard, J.L. (1993). The thyroid gland is a major source of circulating T3 in the rat. J. Clin. Invest. 91, 2709–2713.

Chassande, O., Fraichard, A., Gauthier, K., Flamant, F., Legrand, C., Savatier, P., Laudet, V., and Samarut, J. (1997). Identification of transcripts initiated from an internal promoter in the c-erbA alpha locus that encode inhibitors of retinoic acid receptor-alpha and triiodothyronine receptor activities. Mol. Endocrinol. 11, 1278–1290.

Chédotal, A. (2010). Should I stay or should I go? Becoming a granule cell. Trends Neurosci. 33, 163-172.

Chen, J.D., and Evans, R.M. (1995). A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature. 377, 454–457.

Chiapponi, C., Piras, F., Piras, F., Caltagirone, C., and Spalletta, G. (2016). GABA System in Schizophrenia and Mood Disorders: A Mini Review on Third-Generation Imaging Studies. Front. Psychiatry. 7.

Chiellini, G., Apriletti, J.W., Yoshihara, H.A., Baxter, J.D., Ribeiro, R.C., and Scanlan, T.S. (1998). A high-affinity subtype-selective agonist ligand for the thyroid hormone receptor. Chem. Biol. 5, 299-306.

Cicatiello, A.G., Di Girolamo, D., and Dentice, M. (2018). Metabolic Effects of the Intracellular Regulation of Thyroid Hormone: Old Players, New Concepts. Front Endocrinol (Lausanne) 9,

474.

Columbano, A., Chiellini, G., and Kowalik, M.A. (2017). GC-1: A Thyromimetic With Multiple Therapeutic Applications in Liver Disease. Gene Expr. 17, 265–275.

Cormerais, Y., Giuliano, S., LeFloch, R., Front, B., Durivault, J., Tambutte, E., Massard, P.A., de la Ballina, L.R., Endou, H., et al. (2016). Genetic Disruption of the Multifunctional CD98/LAT1 Complex Demonstrates the Key Role of Essential Amino Acid Transport in the Control of mTORC1 and Tumor Growth. Cancer Res. 76, 4481-4492.

Cortelazzi, D., Morpurgo, P.S., Zamperini, P., Fisher, D.A., Beck-Peccoz, P., and Wu, S.Y. (1999). Maternal compound W serial measurements for the management of fetal hypothyroidsm. Eur. J. Endocrinol. 141, 570–578.

Crantz, F.R. Silva, J.E., Larsen, P.R. (1982). An analysis of the sources and quantity of 3,5,3′-triiodothyronine specifically bound to nuclear receptors in rat cerebral cortex and cerebellum. Endocrinology. 110, 367-375.

D´Agata, F., Ruffinatti, F.A., Boschi, S., Stura, I., Rainero, I., Abollino, O., Cavalli, R., and Guiot, C. (2017). Magnetic Nanoparticles in the Central Nervous System: Targeting Principles, Applications and Safety Issues. Molecules. 23, 1.

Darras, V.M. (2019). The Role of Maternal Thyroid Hormones in Avian Embryonic Development. Front. Endocrinol. 10, 66.

Delange, F. (1997). Neonatal screening for congenital hypothyroidism: results and perspectives. Horm. Res. 48, 51-61.

Delbaere, J., Van Herck, S.L., Bourgeois, N.M., Vancamp, P., Yang, S., Wingate, R.J., and Darras, V.M. (2016). Mosaic Expression of Thyroid Hormone Regulatory Genes defines Cell Type-Specific Dependency in the Developing Chicken Cerebellum. Cerebellum. 15, 710-725.

Delbaere, J., Vancamp, P., Van Herck, S.L., Bourgeios, N.M., Green, M.J., Wingate, R.J., and Darras, V.M. (2017). MCT8 deficiency in Purkinje cells disrupts embryonic chicken cerebellar development. J. Endocrinol. 232, 259-272.

DeLong, G.R., Stanbury, J.B., and Fierro-Benitez, R. (1985). Neurological signs in congenital iodine-deficiency disorder (endemic cretinism). Dev. Med. Child Neurol. 27, 317-324.

Dentice, M., Marsili, A., Zavacki, A., Larsen, P.R., and Salvatore, D. (2013). The deiodinases and the control of intracellular thyroid hormone signaling during cellular differentiation. Biochem. Biophys. Acta. 1830, 3937-3945.

van der Deure, W.M., Hansen, P.S., Peeters, R.P., Kyvik, K.O., Friesema, E.C.H., Hegedüs, L., and Visser, T.J. (2008a). Thyroid hormone transport and metabolism by organic anion transporter 1C1 and consequences of genetic variation. Endocrinology. 149, 5307–5314.

van der Deure, W.M., Appelhof, B.C., Peeters, R.P., Wiersinga, W.M., Wekking, E.M., Huyser, J., Schene, A.H., Tijssen, J.G., Hoogendijk, W.J., Visser, T.J., and Fliers, E. (2008).

Polymorphisms in the brain-specific thyroid hormone transporter OATP1C1 are associated with fatigue and depression in hypothyroid patients. Clin. Endocrinol. 69, 804-811.

Devereaux, J., Ferrara, S.J., Banerji, T., Placzek, A.T., and Scanlan, T.S. (2016). Increasing Thyromimetic Potency through Halogen Substitution. Chem. Med. Chem. 11, 2459–2465.

Devereaux, J., Ferrara, S.J., and Scanlan, T.S. (2018). Quantification of Thyromimetic Sobetirome Concentration in Biological Tissue Samples. Methods Mol. Biol. 1801, 193–206.

Dhar, P., Kaushal, P., and Kumar, P. (2018). Antioxidant supplementation upregulates calbindin expression in cerebellar Purkinje cells of rat pups subjected to post natal exposure to sodium arsenite. Brain Res. 1690, 23–30.

Di Cosmo, C., Liao, X.-H., Dumitrescu, A.M., Weiss, R.E., and Refetoff, S. (2009). A thyroid hormone analog with reduced dependence on the monocarboxylate transporter 8 for tissue transport. Endocrinology. 150, 4450–4458.

Di Cosmo, C., Liao, X.H., Dumitrescu, A.M., Philip, N.J., Weiss, R.E., and Refetoff, S. (2010). Mice deficient in MCT8 reveal a mechanism regulating thyroid hormone secretion. J. Clin. Invest. 120, 3377-3388.

Doran, A., Obach, R.S., Smith, B.J., Hosea, N:A., Becker, S., Callegari, E., Chen, C., Chen, X., Choo, E., Cianfrogna, J., et al. (2005). The impact of P-glycoprotein on the disposition of drugs targeted for indications of the central nervous syste: evaluation using the MDR1A/1B knockout mouse model. Drug Metab. Dispos. 33, 165-174.

Dratman, M.B., Crutchfield, F.L., and Schoenhoff, M.B. (1991). Transport of iodothyronines from bloodstream to brain: contributions by blood: brain and choroid plexus: cerebrospinal fluid barriers. Brain Res. 554, 229-236.

Dremier, S., Milenkovic, M., Blancquaert, S., Dumont, J.E., Doskeland, S.O., Maenhaut, C., and Roger, P.P. (2007). Endocrinology. 148, 4612-4622.

Dumitrescu, A.M., Liao, X.-H., Best, T.B., Brockmann, K., and Refetoff, S. (2004). A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. Am. J. Hum. Genet. 74, 168–175.

Dumitrescu, A.M., Liao, X.H., Weiss, R.E., Millen, K., and Refetoff, S. (2006). Tissue-specific thyroid hormone deprivation and excess in monocarboxylate transporter (mct8) 8-deficient mice. Endocrinology. 147, 4036-4043.

Eayrs, J.T., and Goodhead, B. (1959). Postnatal development of the cerebral cortex in the rat. J. Anat. 93, 385-402.

Egertová, M., Cravatt, B.F., and Elphick, M.R. (2003). Comparative analysis of fatty acid amide hydrolase and cb(1) cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. Neuroscience. 119, 481-496.

Ercan-Fang, S., Schwartz, H.L., and Oppenheimer, J.H. (1996). Isoform-specific 3,5,3′-triiodothyronine receptor binding capacity and messenger ribonucleic acid content in rat adenohypophysis: effect of thyroidal state and comparison with extrapituitary tissues. Endocrinology. 137, 3228-3233.

Eriksson, P.S., Perfilieva, E., Björk-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A., and Gage, F.H. (1998). Neurogenesis in the striatum of the adult human brain. Cell, 156, 1072-1083.

Erlander, M.G., Tillakaratne, N.J., Feldblum, S., Patel, N., and Tobin, A.J. (1991). Two genes encode distinct glutamate decarboxylases. Neuron. 7, 91-100.

Farsetti, A., Desvergne, B., Hallenbeck, P., Robbins, J., and Nikodem, V.M. (1992). Characterization of myelin basic protein thyroid hormone response element and its function in the context of native and heterologous promoter. J. Biol. Chem. 267, 15784–15788.

Fauquier, T., Chatonnet, F., Picou, F., Richard, S., Fossat, N., Aguilera, N., Lamonerie, T., and Flamant, F. (2014). Purkinje cells and Bergmann glia are primary targets of the TRα1 thyroid hormone receptor during mouse cerebellum postnatal development. Development. 141, 166–175.

Fekete, C., and Lechan, R.M. (2007). Negative feedback regulation of hypophysiotropic thyrotropin-releasing hormone (TRH) synthesizing neurons: role of neuronal afferents and type 2 deiodinase. Front Neuroendocrinol. 28, 97–114.

Ferrara, A.M., Liao, X.-H., Gil-Ibáñez, P., Bernal, J., Weiss, R.E., Dumitrescu, A.M., and Refetoff, S. (2014). Placenta passage of the thyroid hormone analog DITPA to male wild-type and Mct8-deficient mice. Endocrinology. 155, 4088–4093.

Ferrara, A.M., Liao, X.H., Ye, H., Weiss, R.E., Dumitrescu, A.M., and Refetoff, S. (2015). The Thyroid Hormone Analog DITPA Ameliorates Metabolic Parameters of Male Mice With Mct8 Deficiency. Endocrinology. 156, 3889-3894.

Ferrara, S.J., Meinig, J.M., Placzek, A.T., Banerji, T., McTigue, P., Hartley, M.D., Sanford-Crane, H.S., Banerji, T., Bourdette, D., and Scanlan, T.S. (2017). Ester-to-amide rearrangement of ethanolamine-derived prodrugs of sobetirome with increased blood-brain barrier penetration. Bioorg. Med. Chem. 25, 2743–2753.

Ferrara, S.J., Bourdette, D., and Scanlan, T.S. (2018). Hypothalamic-Pituitary-Thyroid Axis Pertubations in Male Mice by CNS-Penetrating Thyromimetics. Endocrinology. 259, 2733-2740.

Filgueira, C.S., Nicolov, E., Hood, R.L., Ballerinin, A., Garcia-Huidobro, J., Lin, J.Z., Fraga, D., Webb, P., Sabek, O.M., Gaber, A.O., et al. (2016). Sustained zero-order delivery of GC-1 from a nanochannel membrane device alleviates metabolic syndrome. Int. J. Obes. 40, 1776-1783.

Flamant, F., Poguet, Al.L., Plateroti, M., Chassande, O., Gauthier, K., Streichenberger, N., Mansouri, A., and Samarut, J. (2002). Congenital hypothyroid Pax8(-/-) mutant mice can be

rescued by inactivating the TRalpha gene. Mol. Endocrinol. 16, 24-32.

Flamant, F., and Gauthier, K. (2013). Thyroid hormone receptors: the challenge of elucidating isotype-specific functions and cell-specific response. Biochim. Biophys. Acta. 1830, 3900–3907.

Forrest, D., Erway, L.C., Ng, L., Altschuler, R., and Curran, T. (1996). Thyroid hormone receptor beta is essential for development of auditory function. Nat. Genet. 13, 354-357.

Friedrichsen, S., Christ, S., Heuer, H., Schäfer, M.K.H., Mansouri, A., Bauer, K., and Visser, T.J. (2003). Regulation of iodothyronine deiodinases in the Pax8-/- mouse model of congenital hypothyroidism. Endocrinology. 144, 777–784.

Friesema, E.C., Docter, R., Moerings, E.P., Stieger, B., Hagenbuch, B., Meier, P.J., Krenning, E.P., Hennemann, G., and Visser, T.J. (1999). Identification of thyroid hormone transporters. Biochem. Biophys. Res. Commun. 254, 497-501.

Friesema, E.C., Docter, R., Moerings, E.P., Verrey, F., Krenning, E.P., Hennemann, G., and Visser, T.J. (2001). Thyroid hormone transport by the heterodimeric human system L amino acid transporter. Endocrinology. 142, 4339–4348.

Friesema, E.C.H., Ganguly, S., Abdalla, A., Manning Fox, J.E., Halestrap, A.P., and Visser, T.J. (2003). Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. J. Biol. Chem. 278, 40128–40135.

Friesema, E.C.H., Grueters, A., Biebermann, H., Krude, H., von Moers, A., Reeser, M., Barrett, T.G., Mancilla, E.E., Svensson, J., Kester, M.H.A., et al. (2004). Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. Lancet. 364, 1435–1437.

Friesema, E.C.H., Jansen, J., Heuer, H., Trajkovic, M., Bauer, K., and Visser, T.J. (2006). Mechanisms of disease: psychomotor retardation and high T3 levels caused by mutations in monocarboxylate transporter 8. Nat. Clin. Pract. Endocrinol. Metab. 2, 512–523.

Friesema, E.C.H., Jansen, J., Jachtenberg, J.-W., Visser, W.E., Kester, M.H.A., and Visser, T.J. (2008). Effective cellular uptake and efflux of thyroid hormone by human monocarboxylate transporter 10. Mol. Endocrinol. 22, 1357–1369.

Friesema, E.C., Visser, T.J., Borgers, A.J., Kalsbeek, A., Swaab, D.F., Fliers, E., and Alkemade, A. (2012). Thyroid hormone transporters and deiodinases in the developing human hypothalamus. Eur. J. Endocrinol. 167, 379-386.

Gao, B., Huber, R.D., Wenzel, A., Vavricka, S.R., Ismair, M.G., Reme, C., and Meier, P.J. (2005). Localization of organic anion transporting polypeptides in the rat and human ciliary body epithelium. Exp. Eye Res. 80, 61-72.

Galton, V.A., Wood, E.T., Germain, E.A., Withrow, C.A., Aldrich, G., Germain, G.M., Clark, A.S., Germain, D.L. (2007). Thyroid hormone homeostasis and action in the type 2 deiodinase-deficient rodent brain during development. Endocrinology. 148, 3080-3088.

Genin, E.C., Gondcaille, C., Trompier, D., and Savary, S. (2009). Induction of the adrenoleukodystrophy-related gene (ABCD2) by thyromimetics. J. Steroid Biochem. Mol. Biol. 116, 37-43.

Gereben, B., Zeöld, A., Dentice, M., Salvatore, D., and Bianco, A.C. (2008). Activation and inactivation of thyroid hormone by deiodinases: local action with general consequences. Cell. Mol. Life Sci. 65, 570-590.

Gibson, J.R., Beierlein, M., and Connors, B.W. (1999). Two networks of electrically coupled inhibitory neurons in neocortex. Nature. 402, 75–79.

Gilbert, M.E., Sui, L., Walker, M.J., Anderson, W., Thomas, S., Smoller, S.N., Schon, J.P., Phani, S., and Goodman, J.H. (2007). Thyroid hormone insufficiency during brain development reduces parvalbumin immunoreactivity and inhibitory function in the hippocampus. Endocrinology. 148, 92–102.

Goldman, S., McCarren, M., Morkin, E., Ladenson, P.W., Edson, R., Warren, S., Ohm, J., Thai, H., Churby, L., Barnhill, J., et al. (2009). DITPA (3,5-Diiodothyropropionic Acid), a thyroid hormone analog to treat heart failure: phase II trial veterans affairs cooperative study. Circulation. 119, 3093–3100.

Göthe, S., Wang, Z., Ng, L., Kindblom, J.M., Barros, A.C., Ohlsson, C., Vennström, B., and Forrest, D. (1999). Mice devoid of all known thyroid hormone receptors are viable but exhibit disorders of the pituitary-thyroid axis, growth, and bone maturation. Genes. Dev. 13, 1329-1341.

Groeneweg, S., Visser, W.E., and Visser, T.J. (2017a). Disorder of thyroid hormone transport into the tissues. Best Pract. Res. Clin. Endocrinol. Metab. 31, 241–253.

Groeneweg, S., Peeters, R.P., Visser, T.J., and Visser, W.E. (2017b). Therapeutic applications of thyroid hormone analogues in resistance to thyroid hormone (RTH) syndromes. Mol. Cell. Endocrinol. 458, 82–90.

Groeneweg, S., Peeters, R.P., Visser, T.J., and Visser, W.E. (2017c). Triiodothyroacetic acid in health and disease. J. Endocrinol. 234, R99–R121.

Groeneweg, S., Peeters, R.P., Moran, C., Stoupa, A., Auriol, F., Tonduti, D., Dica, A., Paone, L., Rozenkova, K., Malikova, J., et al., (2019). Effectiveness and safety of the tri-iodothyronine analogue Triac in children and adults with MCT8 deficiency: an international, single-arm, open-label, phase 2 trial. Lancet Diabetes Endocrinol. 7, 695-706.

Gross, J., Pitt-Rivers, R. (1952). The identification of 3:5:3`-L-triiodothyronine in human plasma. Lancet. 1, 439-441.

Grover, G.J., Egan, D.M., Sleph, P.G., Beehler, B.C., Chiellini, G., Nguyen, N.H., Baxter, J.D., and Scanlan, T.S. (2004). Effects of the thyroid hormone receptor agonist GC-1 on metabolic rate and cholesterol in rats and primates: selective actions relative to 3,5,3´-triiodo-L-thyronine. Endocrinology. 145, 1656-1661.

Guadaño-Ferraz, A., Obregón, M.J., St Germain, D.L., and Bernal, J. (1997). The type 2 iodothyronine deiodinase is expressed primarily in glial cells in the neonatal rat brain. Proc. Natl. Acad. Sci. 94, 10391–10396.

Guadaño-Ferraz, A., Escámez, M.J., Rausell, E., and Bernal, J. (1999). Expression of type 2 iodothyronine deiodinase in hypothyroid rat brain indicates an important role of thyroid hormone in the development of specific primary sensory systems. J. Neurosci. 19, 3430–3439.

van Gucht, A.L.M., Moran, C., Meima, M.E., Visser, W.E., Chatterjee, K., Visser, T.J., and Peeters, R.P. (2017). Resistance to Thyroid Hormone due to Heterozygous Mutations in Thyroid Hormone Receptor Alpha. Curr. Top. Dev. Biol. 125, 337–355.

Hagenbuch, B. (2007). Cellular entry of thyroid hormones by organic anion transporting polypeptides. Best Pract. Res. Clin. Endocrinol. Metab. 21, 209-221.

Hajós, F., Patel, A.J., and Balazs, R. (1973). Effect of thyroid deficiency on the synaptic organization of the rat cerebellar cortex. Brain Res. 28, 387-401.

Halestrap, A.P., and Meredith, D. (2004). The SLC16 gene family-from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. Pflugers Arch. 447, 619-628.

Haroutunian, V., Katsel, P., Roussos, P., Davis, K.L., Altshuler, L.L., and Bartzokis, G. (2014). Myelination, oligodendrocytes, and serious mental illness. Glia. 62, 1856–1877.

Hartley, M.D., Kirkemo, L.L., Banerji, T., and Scanlan, T.S. (2017). A Thyroid Hormone-Based Strategy for Correcting the Biochemical Abnormality in X-Linked Adrenoleukodystrophy. Endocrinology. 158, 1328-1338.

Hartley, M.D., Banerji, T., Tagge, I.J., Kirkemo, L.L., Chaudhary, P., Calkins, E., Galipeau, D., Shokat, M.D., et al. (2019). Myelin repair stimulated by CNS-selective thyroid hormone action. JCI Insight. 18, 8.

Hatsukano, T., Kurisu, J., Fukumitsu, K., Fujishima, K., and Kengaku, M. (2017). Thyroid Hormone Induces PGC-1a during Dendritic Outgrowth in Mouse Cerebellar Purkinje Cells. Front. Cell Neurosci. 11, 133.

Hennemann, G., Docter, R., Friesema, E.C., de Jong, M., Krenning, E.P., and Visser, T.J. (2001). Plasma membrane transport of thyroid hormones and its role in thyroid hormone metabolism and bioavailability. Endocr. Rev. 22, 451–476.

van Herck, S.L., Delbaere, J., Bourgeois, N.M., McAllan, B.M., Richardson, S.J., and Darras, V.M. (2015). Expression of thyroid hormone transporters and deiodinases at the brain barriers in the embryonic chicken: Insights into the regulation of thyroid hormone availability during neurodevelopment. Gen. Comp. Endocrinol. 214, 30-39.

Hernandez, A. (2005). Structure and function of the type 3 deiodinase gene. Thyroid. 15, 865-874.

Herzovich, V., Vaiani, E., Marino, R., Dratler, G., Lazzati, J.M., Tilitzky, S., Ramirez, P., Iorcansky, S., Rivarola, M.A., and Belgorosky, A. (2007). Unexpected peripheral markers of thyroid function in a patient with a novel mutation of the MCT8 thyroid hormone transporter gene. Horm. Res. 67, 1-6.

Heuer, H., and Mason, C.A. (2003). Thyroid hormone induces cerebellar Purkinje cell dendritic development via the thyroid hormone receptor alpha1. J. Neurosci. 23, 10604–10612.

Heuer, H., Maier, M.K., Iden, S., Mittag, J., Friesema, E.C., Visser, T.J., and Bauer, K. (2005). The monocarboxylate transporter 8 linked to human psychomotor retardation is highly expressed in thyroid hormone-sensitive neuron populations. Endocrinology. 146, 1701-1706.

Heuer, H., and Visser, T.J. (2009). Minireview: Pathophysiological importance of thyroid hormone transporters. Endocrinology. 150, 1078–1083.

Heuer, H., and Visser, T.J. (2013). The pathophysiological consequences of thyroid hormone transporter deficiencies: Insights from mouse models. Biochim. Biophys. Acta. 1830, 3974–3978.

Heuer, H., Maier, M.K., Iden, S., Mittag, J., Friesema, E.C.H., Visser, T.J., and Bauer, K. (2005). The monocarboxylate transporter 8 linked to human psychomotor retardation is highly expressed in thyroid hormone-sensitive neuron populations. Endocrinology. 146, 1701–1706.

Hitchcock, S.A., and Pennington, L.D. (2006). Structure-brain exposure relationships. J. Med. Chem. 49, 7559–7583.

Hodeshell, K.L. (2002). A model of the development of the brain as a construct of the thyroid system. Environ. Health Perspect. 3, 337-348.

Holden, K.R., Zuñiga, O.F., May, M.M., Su, H., Molinero, M.R., Rogers, R.C., and Schwartz, C.E. (2005). X-linked MCT8 gene mutations: characterization of the pediatric neurologic phenotype. J. Child Neurol. 20, 852–857.

Hörlein, A.J., Näär, A.M., Heinzel, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamei, Y., Söderström, M., and Glass, C.K. (1995). Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature. 377, 397–404.

Horn, S., Kersseboom, S., Mayerl, S., Müller, J., Groba, C., Trajkovic-Arsic, M., Ackermann, T., Visser, T.J., and Heuer, H. (2013). Tetrac can replace thyroid hormone during brain development in mouse mutants deficient in the thyroid hormone transporter mct8. Endocrinology. 154, 968–979.

Huang, Z.J., Di Cristo, G., and Ango, F. (2007). Development of GABA innervation in the cerebral and cerebellar cortices. Nat. Rev. Neurosci. 8, 673–686.

Ibbertson, K., and Fraser, R. (1959). Rapidly acting thyroid hormones and their cardiac action. Br. Med. J. 2, 52–58.

Iwayama, H., Liao, X.H., Braun, L., Barez-López, S., Kaspar, B., Weiss, R.E., Dumitrescu,

A.M., Guadano-Ferraz, A., and Refetoff, S. (2016). Adeno Associated Virus 9-Based Gene Therapy Delivers a Functional Monocarboxylate Transporter 8, Improving Thyroid Hormone Availability to the Brain of Mct8-Deficient Mice. Thyroid. 26, 1311-1319.

Jansen, J. Friesema, E.C., Milici, C., and Visser, T.J. (2005). Thyroid hormone transporters in health and disease. Thyroid. 15, 757-768.

Jiang, M., and Swann, J.W. (1997). Expression of calretinin in diverse neuronal populations during development of rat hippocampus. Neuroscience. 81, 1137–1154.

Johansson, P.A., Dziegielewska, K.M., Liddelow, S.A., and Saunders, N.R. (2008). The blood-CSF barrier explained: when development is not immaturity. Bioessays. 30, 237-248.

Johannsson, G., Svensson, J., and Bengtsson, B.A. (2000). Growth hormone and ageing. Growth Horm. IGF Res. 10, 25-30.

Juge-Aubry, C.E., Morin, O., Pernin, A.T., Liang, H., Philippe, J., and Burger, A.G. (1995). Long-lasting effects of Triac and thyroxine on the control of thyrotropin and hepatic deiodinase type I. Eur. J. Endocrinol. 132, 751-758.

Kallò, I., Mohacsik, P., Vida, B., Zeöld, A., Bardoczi, Z., Zavacki, A., Farkas, E., Kadar, A., Hrabovszky, E., Drigo, R., et al. (2012). A Novel Pathway Regulates Thyroid Hormone Availability in Rat and Human Hypothalamic Neurosecretory Neurons. PLoS One. 7, 6.

Kaplan, M.M., and Yaskoski, K.A. (1980). Phenolic and tyrosyl ring deiodination of iodothyronines in rat brain homogenates. J. Clin. Invest. 66, 551–562.

Katsumaru, H., Kosaka, T., Heizmann, C.W., and Hama, K. (1988). Immunocytochemical study of GABAergic neurons containing the calcium-binding protein parvalbumin in the rat hippocampus. Exp. Brain Res. 72, 247-362.

Kaufman, D.L., Houser, C.R., and Tobin, A.J. (1991). Two forms of the gamma-aminobutyric acid synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions. J. Neurochem. 56, 720–723.

Kersseboom, S., and Visser, T.J. (2011). MCT8: from gene to disease and therapeutic approach. Ann. Endocrinol. 72, 77-81.

Kersseboom, S., Horn, S., Visser, W.E., Chen, J., Friesema, E.C.H., Vaurs-Barrière, C., Peeters, R.P., Heuer, H., and Visser, T.J. (2014). In vitro and mouse studies supporting therapeutic utility of triiodothyroacetic acid in MCT8 deficiency. Mol. Endocrinol. 28, 1961–1970.

Kimura-Kuroda, J., Nagata, I., Negishi-Kato, M., and Kuroda, Y. (2002). Thyroid hormone-dependent development of mouse cerebellar Purkinje cells in vitro. Brain Res. Dev. Brain Res. 137, 55–65.

Kobayashi, Y. (2007). Localization of the System L Amino Acid Transporters LAT1 and LAT2 in Rat Gastrointestinal Tract. J. Kyorin Med. Soci. 38, 75–84.

Kobayashi, N., Okazaki, S., Sampetrean, O., Irie, J., Itoh, H., and Saya, H. (2018). CD44 variant

inhibits insulin secretion in pancreatic ß cells by attenuating LAT1-mediated amino acid uptake. Sci. Rep. 8, 2785.

Köhrle, J. (1990). Thyrotropin (TSH) action on thyroid hormone deiodination and secretion: one aspect of thyrotropin regulation of thyroid cell biology. Horm. Metab. Res. 23, 18-28.

Koibuchi, N. (2008). The role of thyroid hormone on cerebellar development. Cerebellum 7, 530–533.

Koibuchi, N., and Chin, W.W. (2000). Thyroid hormone action and brain development. Trends Endocrinol. Metab. 11, 123-128.

Krassas, G.E, Rivkees, S.A, Kiess, W. (2007). Diseases of the Thyroid in Childhood and Adolescence. (Basel: Karger).

Lafreniére, R.G., Carrel, L., and Willard, H.F. (1994). A novel transmembrane transporter encoded by the XPCT gene in Xq13.2. Hum. Mol. genet. 3, 1133-1139.

Lameloise, N., Siegrist-Kaiser, C., O'Connell, M., and Burger, A. (2001). Differences between the effects of thyroxine and tetraiodothyroacetic acid on TSH suppression and cardiac hypertrophy. Eur. J. Endocrinol. 144, 145–154.

Lauder, J.M. (1977). The effects of early hypo- and hyperthyroidism on the development of rat cerebellar cortex. III. Kinetics of cell proliferation in the external granular layer. Brain Res. 126, 31–51.

Lazar, M.A. (1993). Thyroid hormone receptors: multiple forms, multiple possibilities. Endocr. Rev. 14, 184–193.

Le Magueresse, C., and Monyer, H. (2013). GABAergic interneurons shape the functional maturation of the cortex. Neuron 77, 388–405.

Lechan, R.M., Qi, Y., Jackson, I.M., and Mahdavi, V. (1994). Identification of thyroid hormone receptor isoforms in thyrotropin-releasing hormone neurons of the hypothalamic paraventricular nucleus. Endocrinology. 135, 92–100.

Lee, J.Y., and Petratos, S. (2016). Thyroid Hormone Signaling in Oligodendrocytes: from Extracellular Transport to Intracellular Signal. Mol. Neurobiol. 53, 6568–6583.

Lee, J.Y, Kim, M.J., Deliyanti, D., Azari, M.F., Rossello, F., Costin, A., Ramm, G., Stanley, E.G., Elefanty, A.G., et al. (2017). Overcoming Monocarboxylate Transporter 8 (MCT8)-Deficiency to Promote Human Oligodendrocyte Differentiation and Myelination. EBioMedicine. 25, 122-135.

Legrand, J. (1984). Effects of thyroid hormones on central nervous system. In: Yanai J (eds) Neurobehavioral Teratology. Elsevier, Amsterdam, 331-363.

Legrand, J., Selme-Matrat, M., Rabie, A., Clos, J., Legrand, C. (1976). Thyroid hormone and cell formation in the developing rat cerebellum. Biol. Neonate. 29, 368-380.

Leitch, V.D., Di Cosmo, C., Liao, X.H., O'Boy, S., Galliford, T.M., Evans, H., Croucher, P.I., Boyde, A., Dumitrescu, A., et al. (2017). An Essential Physiological Role for MCT8 in Bone in Male Mice. Endocrinology. 158, 3055-3066.

Liang, H., Juge-Aubry, C.E., O'Connell, M., and Burger, A.G. (1997). Organ-specific effects of 3,5,3'-triiodothyroacetic acid in rats. Eur. J. Endocrinol. 137, 537-544.

Liao, X.H., Di Cosmo, C., Dumitrescu, A.M., Hernandez, A., Van Sande, J., Germain, D.L., Weiss, R.E., Galton, V.A., and Refetoff, S. (2011). Distinct roles of deiodinases on the phenotype of Mct8 defect: a comparison of eight different mouse genotypes. Endocrinology. 152, 1180-1191.

Lin, J.Z., Martagon, A.J., Hsueh, W.A., Baxter, J.D., Gustafsson, J.A., Webb, P., Phillips, K.J. (2012). Thyroid hormone receptor agonists reduce serum cholesterol independent of the LDL receptor. Endocrinology. 153, 6136-6144.

López-Espíndola, D., Morales-Bastos, C., Grijota-Martínez, C., Liao, X.-H., Lev, D., Sugo, E., Verge, C.F., Refetoff, S., Bernal, J., and Guadaño-Ferraz, A. (2014). Mutations of the thyroid hormone transporter MCT8 cause prenatal brain damage and persistent hypomyelination. J. Clin. Endocrinol. Metab. 99, 2799-2804.

López-Espíndola, D., Garcia-Aldea, A., Gómez de la Riva, I., Rodriguez-Garcia, A.M., Salvatore, D., Visser, T.J., Bernal, J., and Guadaño-Ferraz, A. (2019). Thyroid hormone availability in the human fetal brain: novel entry pathways and role of radial glia. Brain Struct. Funct. 224, 2103-2119.

Mansouri, A., Chowdhury, K., and Gruss, P. (1998). Follicular cells of the thyroid gland require Pax8 gene function. Nat. Genet. 19, 87-90.

Manzano, J., Cuadrado, M., Morte, B., and Bernal, J. (2007). Influence of thyroid hormone and thyroid hormone receptors in the generation of cerebellar gamma-aminobutyric acid-ergic interneurons from precursor cells. Endocrinology. 148, 5746–5751.

Marín, O., and Müller, U. (2014). Lineage origins of GABAergic versus glutamatergic neurons in the neocortex. Curr. Opin. Neurobiol. 26, 132–141.

Marín-Padilla, M. (1990). Three-dimensional structural organization of layer I of the human cerebral cortex: a Golgi study. J. Comp. Neurol. 299, 89–105.

Mariotta, L., Ramadam, T., Singer, D., Guetg, A., Herzog, B., Stoeger, C., Palacin, M., Lahoutte, T., Camargo, S.M., and Verrey, F. (2012). T-type amino acid transporter TAT1 (Slc16a10) is essential for extracellular aromatic amino acid homeostasis control. J. Physiol. 590, 6413-6424.

Martagón, A.J., Lin, J.Z., Cimini, S.L., Webb, P., and Phillips, K.J. (2015). The amelioration of hepatic steatosis by thyroid hormone receptor agonists is insufficient to restore insulin sensitivity in ob/ob mice. PLoS One. 10, 4.

Mastroberardino, L., Spindler, B., Pfeiffer, R., Skelly, P.J., Loffing, J., Shoemaker, C.B., and

Verrey, F. (1998). Amino-acid transport by heterodimers of 4F2hc/CD98 and members of a permease family. Nature 395, 288–291.

Mayerl, S., Visser, T.J., Darras, V.M., Horn, S., and Heuer, H. (2012). Impact of Oatp1c1 deficiency on thyroid hormone metabolism and action in the mouse brain. Endocrinology. 153, 1528–1537.

Mayerl, S., Müller, J., Bauer, R., Richert, S., Kassmann, C.M., Darras, V.M., Buder, K., Boelen, A., Visser, T.J., and Heuer, H. (2014). Transporters MCT8 and OATP1C1 maintain murine brain thyroid hormone homeostasis. J. Clin. Invest. 124, 1987–1999.

Mayerl, S., Schmidt, M., Doycheva, D., Darras, V.M., Hüttner, S.S., Boelen, A., Visser, T.J., Kaether, C., Heuer, H., and von Maltzahn, J. (2018). Thyroid Hormone Transporters MCT8 and OATP1C1 Control Skeletal Muscle Regeneration. Stem Cell Reports 10, 1959–1974.

McKenna, N.J., and O'Malley, B.W. (2002). Minireview: nuclear receptor coactivators--an update. Endocrinology. 143, 2461–2465.

McPartland, J., and Volkmar, F.R. (2012). Autism and related disorders. Handb. Clin. Neurol. 106, 407-418.

Meinig, J.M., Ferrara, S.J., Banerji, T., Banerji, T., Sanford-Crane, H.S., Bourdette, D., and Scanlan, T.S. (2017). Targeting Fatty-Acid Amide Hydrolase with Prodrugs for CNS-Selective Therapy. ACS Chem. Neurosci. 8, 2468–2476.

Meinig, J.M., Ferrara, S.J., Banerji, T., Banerji, T., Sanford-Crane, H.S., Bourdette, D., and Scanlan, T.S. (2019). Structure-Activity Relationships of Central Nervous System Penetration by Fatty Acid Amide Hydrolase (FAAH)-Targeted Thyromimetic Prodrugs. ACS Med. Chem. Lett. 10, 111–116.

Menegay, C., Juge, C., and Burger, A.G. (1989). Pharmacokinetics of 3,5,3′-triiodothyroacetic acid and its effects on serum TSH levels. Acta Endocrinol. 121, 651-658.

Messier, N., and Langlois, M.F. (2000). Triac regulation of transcription is T(3) receptor isoform- and response element-specific. Mol. Cell Endocrinol. 165, 57-66.

Ming, G.-L., and Song, H. (2011). Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron. 70, 687–702.

Mirell, C.J., Yanagisawa, M., and Hershman, J.M. (1989). Triac reduces serum TSH without decreasing alpha and beta TSH messenger RNAs. Horm. Metab. Res. 21, 123-126.

Mohan, V., Sinha, R.A., Pathak, A., Rastogi, L., Kumar, P., Pal, A., and Godbole, M.M. (2012). Maternal thyroid hormone deficiency affects the fetal neocorticogenesis by reducing the proliferating pool, rate of neurogenesis and indirect neurogenesis. Exp. Neurol. 237, 477–488.

Moog, N.K., Entringer, S., Heim, C., Wadhwa, P.D., Kathmann, N., and Buss, C. (2017). Influence of maternal thyroid hormones during gestation on fetal brain development. Neuroscience. 342, 68–100.

Morte, B., Manzano, J., Scanlan, T.S., Vennström, B., and Bernal, J. (2004). Aberrant maturation of astrocytes in thyroid hormone receptor alpha 1 knockout mice reveals an interplay between thyroid hormone receptor isoforms. Endocrinology. 145, 1386-1391.

van Mullem, A.A., Chrysis, D., Eythimiadou, A., Chroni, E., Tsatsoulis, A., de Rijke, Y.B., Visser, W.E., Visser, T.J., and Peeters, R.P. (2013). Clinical phenotype of a new type of thyroid hormone resistance caused by a mutation of the TR alpha1 receptor: consequences of LT4 treatment. J. Clin. Endocrinol. Metab. 98, 3029-3038.

Müller, J., and Heuer, H. (2014). Expression pattern of thyroid hormone transporters in the postnatal mouse brain. Front Endocrinol. 5, 92.

Müller, J., Mayerl, S., Visser, T.J., Darras, V.M., Boelen, A., Frappart, L., Mariotta, L., Verrey, F., and Heuer, H. (2014). Tissue-specific alterations in thyroid hormone homeostasis in combined Mct10 and Mct8 deficiency. Endocrinology. 155, 315–325.

Müller-Gärtner, H.W., and Schneider, C. (1988). 3,5,3′-triiodothyroacetic acid minimize the pituitary thyrotrophin secretion in patients on levo-thyroxine therapy after ablative therapy for differentiated thyroid carcinoma. Clin. Endocrinol. 28, 345.

Nag, T.C., and Wadhwa, S. (1999). Calbindin immunoreactivity in the developing and adult human cerebellum. J. Chem. Neuroanat. 17, 1–12.

Nakada, N., Mikami, T., Hana, K., Ichinoe, M., Yanagisawa, N., Yoshida, T., Endou, H., and Okayasu, I. (2014). Unique and selective expression of L-amino acid transporter 1 in human tissue as well as being an aspect of oncofetal protein. Histol. Histopathol. 29, 217–227.

Nicholson, J.L., and Altman, J. (1972). Synaptogenesis in the rat cerebellum: effects of early hypo- and hyperthyroidism. Science. 176, 530-532.

Nikrodhanond, A.A., Ortiga-Carvalho, T.M., Shibusawa, N., Hashimoto, K., Liao, X.H., Refetoff, S., Yamada, M., Mori, M., and Wondisford, F.E. (2006). Dominant role of thyrotropin-releasing hormone in the hypothalamic-pituitary-thyroid axis. J. Biol. Chem. 281, 5000-5007.

Nishimura, M., and Naito, S. (2008). Tissue-specific mRNA Expression Profiles of Human Solute Carrier Transporter Superfamilies. Drug Metab. Pharmacokinet. 23, 22-44.

Novara, F., Groeneweg, S., Freri, E., Estienne, M., Reho, P., Matricardi, S., Castelotti, B., Visser, W.E., Zuffardi, O., and Visser, T.J. (2017). Clinical and Molecular Characteristics of SLC16A2 (MCT8) Mutations in Three Families with the Allan-Herndon-Dudley Syndrome. Hum. Mutat. 38, 260-264.

Núñez, B., Martínez de Mena, R., Obregon, M.J., Font-Llitjós, M., Nunes, V., Palacín, M., Dumitrescu, A.M., Morte, B., and Bernal, J. (2014). Cerebral cortex hyperthyroidism of newborn mct8-deficient mice transiently suppressed by lat2 inactivation. PLoS ONE 9, 96915.

Obermeier, B., Daneman, R., and Ransohoff, R.M. (2013). Development, maintenance and disruption of the blood-brain barrier. Nat. Med. 19, 1584–1596.

Oliver, M.F., and Boyd, G.S. (1957). Effect of phenylethylacetic acid and its amide (hyposterol) on the circulating lipids and lipoproteins in man. Lancet. 273, 829–830.

Oppenheimer, J.H., and Schwartz, H.L. (1997). Molecular basis of thyroid hormone-dependent brain development. Endocr. Rev. 18, 462–475.

Ortiga-Carvalho, T.M., Chiamolera, M.I., Pazos-Moura, C.C., and Wondisford, F.E. (2016). Hypothalamus-Pituitary-Thyroid Axis. Compr. Physiol. 6, 1387–1428.

O'Shaughnessy, K.L., Thomas, S.E., Spring, S.R., Ford, J.L., Ford, R.L., and Gilbert, M.E. (2019). A transient window of hypothyroidism alters neural progenitor cells and results in abnormal brain development. Sci. Rep. 9, 4662.

Palacin, M. (1994). A new family of proteins (rBAT and 4F2hc) involved in cationic and zwitterionic amino acid transport: a tale of two proteins in search of a transport function. J. Exp. Biol. 196, 123-137.

Pardridge, W.M. (1979). Carrier-mediated transport of thyroid hormones through the rat blood-brain barrier: primary role of albumin-bound hormone. Endocrinology. 105, 605–612.

Pasquini, J.M., and Adamo, A.M. (1994). Thyroid hormones and the central nervous system. Dev. Neurosci. 16, 1–8.

Peng, Y.-R., He, S., Marie, H., Zeng, S.-Y., Ma, J., Tan, Z.-J., Lee, S.Y., Malenka, R.C., and Yu, X. (2009). Coordinated changes in dendritic arborization and synaptic strength during neural circuit development. Neuron. 61, 71–84.

Pennock, G.D., Raya, T.E., Bahl, J.J., Goldman, S., and Morkin, E. (1992). Cardiac effects of 3,5-diiodothyropropionic acid, a thyroid hormone analog with inotropic selectivity. J. Pharmacol. Exp. Ther. 263, 163-169.

Pennock, G.D., Raya, T.E., Bahl, J.J., Goldman, S., Morkin, E. (1993). Combination treatment with captopril and the thyroid hormone analogue 3,5-diiodothyropropionic acid. A new approach to improving left ventricular performance in heart failure. Circulation. 88, 1289-1298.

Perra, A., Simbula, G., Simbula, M., Pibiri, M., Kowalik, M.A., Sulas, P., Cocco, M.T., Ledda-Columbano, G.M., and Columbano, A. (2008). Thyroid hormone (T3) and TRbeta agonist GC-1 inhibit/reverse nonalcoholic fatty liver in rats. FASEB J. 22, 2981-2989.

Petilla Interneuron Nomenclature Group, Ascoli, G.A., Alonso-Nanclares, L., Anderson, S.A., Barrionuevo, G., Benavides-Piccione, R., Burkhalter, A., Buzsáki, G., Cauli, B., Defelipe, J., et al. (2008). Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. Nat. Rev. Neurosci. 9, 557–568.

Pierre, K., and Pellerin, L. (2005). Monocarboxylate transporters in the central nervous system: distribution, regulation and function. J. Neurochem. 94, 1–14.

Pinho, M.J., Gomes, P., Serrão, M.P., Bonifácio, M.J., and Soares-da-Silva, P. (2003). Organ-specific overexpression of renal LAT2 and enhanced tubular L-DOPA uptake precede the onset

of hypertension. Hypertension. 42, 613–618.

Pinho, M.J., Serrão, M.P., Gomes, P., Hopfer, U., Jose, P.A., and Soares-da-Silva, P. (2004). Over-expression of renal LAT1 and LAT2 and enhanced L-DOPA uptake in SHR immortalized renal proximal tubular cells. Kidney Int. 66, 216–226.

Pizzagalli, F., Hagenbuch, B., Stieger, B., Klenk, U., Folkers, G., and Meier, P.J. (2002). Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter. Mol. Endocrinol. 16, 2283-2296.

Placzek, A.T., and Scanlan, T.S. (2015). New synthetic routes to thyroid hormone analogs:d_6-sobetirome, ^{3}H-sobetirome and the antagonist NH-3. Tetrahedron. 71, 5946-5951.

Placzek, A.T., Ferrara, S.J., Hartley, M.D., Sanford-Crane, H.S., Meinig, J.M., and Scanlan, T.S. (2016). Sobetirome prodrug esters with enhanced blood-brain barrier permeability. Bioorg. Med. Chem. 24, 5842–5854.

Portella, A.C., Carvalho, F., Faustino, L., Wondisford, F.E., Ortiga-Carvalho, T.M., and Gomes, F.C.A. (2010). Thyroid hormone receptor beta mutation causes severe impairment of cerebellar development. Mol. Cell. Neurosci. 44, 68–77.

Price, N.T., Jackson, V.N., and Halestrap, A.P. (1998). Cloning and sequnecing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. Biochem. J. 329, 321-328.

Pugeat, M., Crave, J.C., Tourniaire, J., and Forest, M.G. (1996). Clinical utility of sex hormone-binding globulin measurment. Horm. Res. 45, 148-155.

Radetti, G., Persani, L., Molinaro, G., Mannavola, D., Cortelazzi, D., Chatterjee, V.K., Beck-Peccoz, P. (1997). Clinical and hormonal outcome after two years of triiodothyroacetic treatment in a child with thyroid hormone resistance. Thyroid. 7, 775-778.

Ramadam, T., Camargo, S.M., Summa, V., Hunziker, P., Chesnov, S., Pos, K.M., and Verrey, F. (2006). Basolateral aromatic amino acid transporter TAT1 (Slc16a10) functions as an efflux pathway. J. Cell Physiol. 206, 771-779.

Ramadam, T., Camargo, S.M., Herzog, B., Bordin, M., Pos, K.M., and Verrey, F. (2007). Recycling of aromatic amino acids via TAT1 allows efflux of neutral amino acids via LAT2-4F2hc exchanger. Pflugers Arch. 454, 507-516.

Rautio, J., Laine, K., Gynther, M., and Savolainen, J. (2008). Prodrug approaches for CNS delivery. AAPS J. 10, 92–102.

Refetoff, S., Weiss, R.E., and Usala, S.J. (1993). The syndrome of resistance to thyroid hormone. Endocr. Rev. 14, 348-399.

Richard, S., Guyot R., Rey-Millet M., Prieux M., Markossian S., Aubert D., and Flamant F. (2019). A pivotal genetic program controlled by thyroid hormone during the maturation of GABAergic neurons in mice. iScience. 23, 100899.

del Río, J.A., de Lecea, L., Ferrer, I., and Soriano, E. (1994). The development of parvalbumin-immunoreactivity in the neocortex of the mouse. Brain Res. Dev. Brain Res. 81, 247–259.

Roberts, L.M., Woodford., K., Zhou, M., Black, D.S., Haggerty, J.E., Tate, E.H., Grindstaff, K.K., Mengesha, W., Raman, C., and Zerangue, N. (2008). Expression of the thyroid hormone transporters monocarboxylate transporter-8 (SLC16A2) and organic ion transporter-14 (SLCO1C1) at the blood brain barrier. Endocrinology. 149, 6251-6261.

Rodríguez-Peña, A. (1999). Oligodendrocyte development and thyroid hormone. J. Neurobiol. 40, 497–512.

Rodríguez-Peña, A. (1993). Neonatal hypothyroidism affects the timely expression of myelin-associated glycoprotein in the rat brain. J. Clin. Invest. 91, 812-818.

Rosario, F.J., Janson, N., Kanai, Y., Prasad, P.D., Powell, T.L., and Jansson, T. (2011). Maternal protein restriction in the rat inhibits placental insulin, mTOR, and STAT3 signaling and down-regulates placental amino acid transporters. Endocrinology. 152, 1119-1129.

Rudy, B., Fishell, G., Lee, S., and Hjerling-Leffler, J. (2011). Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. Dev. Neurobiol. 71, 45–61.

Scalise, M., Galluccio, M., Console, L., Pochini, L., and Indiveri, C. (2018). The Human SLC7A5 (LAT1): The Intriguing Histidine/Large Neutral Amino Acid Transporter and Its Relevance to Human Health. Front. Chem. 6, 243.

Scanlan, T.S. (2010). Sobetirome: a case history of bench-to-clinic drug discovery and development. Heart Fail Rev. 15, 177–182.

Schwartz, H.L., Strait, K.A., Ling, N.C., and Oppenheimer, J.H. (1992). Quantitation of rat tissue thyroid hormone binding receptor isoforms by immunoprecipitation of nuclear triiodothyronine binding capacity. J. Biol. Chem. 267, 11794-11799.

Schwartz, C.E., and Stevenson, R.E. (2007). The MCT8 thyroid hormone transporter and Allan-Herndon-Dudley syndrome. Best Pract. Res. Clin. Endocrinol. Metab. 21, 307–321.

Schwartz, C.E., May, M.M., Carpenter, N.J., Rogers, R.C., Martin, J., Bialer, M.G., Ward, J., Sanabria, J., Marsa, S., Lewis, J.A., et al. (2005). Allan-Herndon-Dudley syndrome and the monocarboxylate transporter 8 (MCT8) gene. Am. J. Hum. Genet. 77, 41–53.

Sijens, P.E., Rödiger, L.A., Meiners, L.C., and Lunsing, R.J. (2008). 1H magnetic resonance spectroscopy in monocarboxylate transporter 8 gene deficiency. J. Clin. Endocrinol. Metab. 93, 1854-1859.

Shaji, D. (2018). Molecular docking studies of human MCT8 protein with soy isoflavones in Allan-Herndon-Dudley syndrome (AHDS). J. Pharm. Anal. 8, 318–323.

Shepro, D. and Morel, N.M. (1993). Pericyte physiology. FASEB J. 11, 1031-1038.

Soghomonian, J.J., and Martin, D.L. (1998). Two isoforms of glutamate decarboxylase: why? Trends Pharmacol. Sci. 19, 500–505.

Sreenivasan, V., Esmaeili, V., Kiritani, T., Galan, K., Crochet, S., and Petersen, C.C.H. (2016). Movement Initiation Signals in Mouse Whisker Motor Cortex. Neuron. 92, 1368–1382.

Strait, K.A., Carlson, D.J., Schwartz, H.L., and Oppenheimer, J.H. (1997). Transient stimulation of myelin basic protein gene expression in differentiating cultured oligodendrocytes: a model for 3,5,3'-triiodothyronine-induced brain development. Endocrinology. 138, 635–641.

Strait, K.A., Schwartz, H.L., Seybold, V.S., Ling, N.C., and Oppenheimer, J.H. (1991). Immunofluorescence localization of thyroid hormone receptor protein beta 1 and variant alpha 2 in selected tissues: cerebellar Purkinje cells as a model for beta 1 receptor-mediated developmental effects of thyroid hormone in brain. Proc. Natl. Acad. Sci. 88, 3887-3891.

Strømme, P., Groeneweg, S., Lima de Souza, E.C., Zevenbergen, C., Torgersbråten, A., Holmgren, A., Gurcan, E., Meima, M.E., Peeters, R.P., Visser, W.E., et al. (2018). Mutated Thyroid Hormone Transporter OATP1C1 Associates with Severe Brain Hypometabolism and Juvenile Neurodegeneration. Thyroid. 28, 1406–1415.

Sugiyama, D., Kusuhara, H., Taniguchi, H., Ishikawa, S., Nozaki, Y., Aburatani, H., and Sugiyama, Y. (2003). Functional characterization of rat brain-specific organic anion transporter (Oatp14) at the blood-brain barrier: high affinity transporter for thyroxine. J. Biol. Chem. 31, 43489-43495.

Talukder, M.A., Yang, F., Nishijima, Y., Chen, C.A., Xie, L., Mahamud, S.D., Kalyanasundaram, A., et al. (2010). Detrimental effects of thyroid hormone analog DITPA in the mouse heart: increased mortality with in vivo acute myocardial ischemia-reperfusion. Am. J. Physiol. Heart Circ. Physiol. 300, 702-711.

Tancevski, I., Eller, P., Pathsc, J.R., and Ritsch, A. (2009). The resurgence of thyromimetics as lipid-modifying agents. Curr. Opin. Investig. Drugs. 10, 912-918.

Takeda, T., Suzuki, S., Liu, R.T., and DeGroot, L.J. (1995). Triiodothyroacetic acid has unique potential for therapy of resistance to thyroid hormone. J. Clin. Endocrinol. Metab. 80, 2033–2040.

Thompson, C.C., and Potter, G.B. (2000). Thyroid hormone action in neural development. Cereb. Cortex. 10, 939–945.

Tinnikov, A., Nordström, K., Thoren, P., Kindblom, J.M., Malin, S., Rozell, B., Adams, M., Rajanayagam, O., Pettersson, S., Ohlsson, C., Chatterjee, K., and Vennström, B. (2002). Retardation of post-natal development caused by a negatively acting thyroid hormone receptor alpha1. EMBO. J. 21, 5079-5087.

Tohyama, K., Kusuhara, H., and Sugiyama, Y. (2004). Involvement of multispecific organic anion transporter, Oatp14 (Slc21a14), in the transport of thyroxine across the blodd-brain barrier. Endocrinology. 145, 4384-4391.

Tonduti, D., Vanderver, A., Berardinelli, A., Schmidt, J.L., Collins, C.D., Novara, F., Genni, A.D., Mita, A., Triulzi, F., Brunstrom-Hernandez, J.E., et al. (2013). MCT8 deficiency:

extrapyramidal symptoms and delayed myelination as prominent features. J. Child Neurol. 28, 795–800.

Trajkovic, M., Visser, T.J., Mittag, J., Horn, S., Lukas, J., Darras, V.M., Raivich, G., Bauer, K., and Heuer, H. (2007). Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8. J. Clin. Invest. 117, 627–635.

Trajkovic-Arsic, M., Visser, T.J., Darras, V.M., Friesema, E.C., Schlott, B., Mittag, J., Bauer, K., and Heuer, H. (2010). Consequneces of monocarboxylate transporter 8 deficiency for renal transport and metabolism of thyroid hormones in mice. Endocrinology. 151, 802-809.

Trost, S.U., Swanson, E., Gloss, B., Wang-Iverson, D.B., Zhang, H., Volodarsky, T., Grover, G.J., Baxter, J.D., Chiellini, G., Scanlan, T.S., and Dillmann, W.H. (2000). The thyroid hormone receptor-beta-selective agonist GC-1 differentially affects plasma lipids and cardiac activity. Endocrinology. 141, 3057-3064.

Tsou, K., Nogueron, M.I., Muthian, S., Sañudo-Pena, M.C., Hillard, C.J., Deutsch, D.G., and Walker, J.M. (1998). Fatty acid amide hydrolase is located preferentially in large neurons in the rat central nervous system as revealed by immunohistochemistry. Neurosci. Lett. 254, 137–140.

Tu, H.M., Kim, S.W., Salvatore, D., Bartha, T., Legradi, G., Larsen, P.R., and Lechan, R.M. (1997). Regional distribution of type 2 thyroxine deiodinase messenger ribonucleic acid in rat hypothalamus and pituitary and its regulation by thyroid hormone. Endocrinology. 138, 3359-3368.

Tu, H.M., Legradi, G., Bartha, T., Salvatore, D., Lechan, R.M., and Larsen, P.R. (1999). Regional expression of the type 3 iodothyronine deiodinase messenger ribonucleic acid in the rat central nervous system and its regulation by thyroid hormone. Endocrinology. 140, 784–790.

Uchida, K., Taguchi, Y., Sato, C., Miyazaki, H., Kobayashi, K., Kobayashi, T., and Itoi, K. (2014). Amelioration of improper differentiation of somatostatin-positive interneurons by triiodothyronine in a growth-retarded hypothyroid mouse strain. Neurosci. Lett. 559, 111–116.

Vancamp, P., and Darras, V.M. (2017). Dissecting the role of regulators of thyroid hormone availability in early brain development: Merits and potential of the chicken embryo model. Mol. Cell Endocrinol. 25, 71-78.

Vann, S.D., Aggleton, J.P., and Maguire, E.A. (2009). What does the retrosplenial cortex do? Nat. Rev. Neurosci. 10, 792–802.

Vaidya, C.J. (2012). Neurodevelopmental abnormalities in ADHD. Curr. Top Behav. Neurosci. 9, 49-66.

Vasilopoulou, E. Loubiere, L.S., Heuer, H., Trajkovic-Arsic, M., Darras, V.M., Visser, T.J., Lash, G.E., Whitley, G.S., McCabe, C.J., Franklyn, J.A., Kilby, M.D., and Chan, S.Y. (2013). Monocarboxylate transporter 8 modulates the viability and invasive capacity of human placental cells and fetoplacental growth in mice. PLoS One. 8, 6.

Vatine, G.D., Zada, D., Lerer-Golshtein, T., Tovin, A., Malkinsons, G., Yaniv, K., and Appelbaum, L. (2013). Zebrafish as a model for monocarboxyl transporter 8-deficiency. J. Biol. Chem. 288, 169-180.

Vatner, D.F., Weismann, D., Beddow, S.A., Kumashiro, N., Erion, D.M., Liao, X.H., Grover, G.J., Webb, P., Phillips, K.J., et al. (2013). Thyroid hormone receptor-beta agonists prevent hepatic steatosis in fat-fed rats but impair insulin sensitivity via discrete pathways. Am. J. Physiol. Endocrinol. Metab. 305, 89-100.

Verge, C.F., Konrad, D., Cohen, M., Di Cosmo, C., Dumitrescu, A.M., Marcinkowski, T., Hameed, S., Hamilton, J., Weiss, R.E., and Refetoff, S. (2012). Diiodothyropropionic acid (DITPA) in the treatment of MCT8 deficiency. J. Clin. Endocrinol. Metab. 97, 4515–4523.

Verrey, F., Jack, D.L., Paulsen, I.T., Saier, M.H., and Pfeiffer, R. (1999). New glycoprotein-associated amino acid transporters. J. Membr. Biol. 172, 181–192.

Vicente, N., Cardoso, L., Barros, L., Carrilho, F. (2017). Antithyroid drug-induced agranulocytosis: state of the art on diagnosis and management. Drugs. 17, 91-96.

Vincent, J., Legrand, C., Rabié, A., and Legrand, J. (1982). Effects of thyroid hormone on synaptogenesis in the molecular layer of the developing rat cerebellum. J. Physiol. (Paris) 78, 729–738.

Virgili, M., Contestabile, A., and Barnabei, O. (1991). Postnatal maturation of cholinergic markers in forebrain regions of C57BL/6 mice. Brain Res. Dev. Brain Res. 63, 281–285.

Visser, W.E., Friesema, E.C.H., Jansen, J., and Visser, T.J. (2008). Thyroid hormone transport in and out of cells. Trends Endocrinol. Metab. 19, 50–56.

Visser, W.E., Jansen, J., Friesema, E.C., Kester, M.H., Mancilla, E., Lundgren, J., van der Knaap, M.S., Lunsing, R.J., Brouwer, O.F., and Visser, T.J. (2009). Novel pathogenic mechanism suggested by ex vivo analysis of MCT8 (SLC16A2) mutations. Hum. Mutat. 30, 29-38.

Visser, W.E., Friesema, E.C.H., and Visser, T.J. (2011). Minireview: thyroid hormone transporters: the knowns and the unknowns. Mol. Endocrinol. 25, 1–14.

Visser, W.E., Visser, T.J., and Peeters, R.P. (2013). Thyroid disorders in older adults. Endocrinol. Metab. Clin. North Am. 42, 287–303.

Visser, W.E., Heuer, H., and Visser, T.J. (2016). Triiodothyroacetic Acid Treatment in MCT8 Deficiency: A Word of Nuance. Thyroid. 26, 615–617.

de Vrieze, E., van de Wiel, S.M., Zethof, J., Filk, G., Klaren, P.H., and Arjona, F.J. (2014). Knockdown of monocarboxylate transporter 8 (mct8) disturbs brain development and locomotion in zebrafish. Endocrinology. 155, 2320-2330.

Walker, D.M., Kirson, D., Perez, L.F., and Gore, A.C. (2012). Molecular profiling of postnatal development of the hypothalamus in female and male rats. Biol. Reprod. 87, 129.

Wang, Q., and Holst, J. (2015). L-type amino acid transport and cancer: targeting the mTORC1 pathway to inhibit neoplasia. Am. J. Cancer Res. 5, 1281-1294.

Wallis, K., Sjögren, M., van Hogerlinden, M., Silberberg, G., Fisahn, A., Nordström, K., Larsson, L., Westerblad, H., Morreale de Escobar, G., Shupliakov, O., et al. (2008). Locomotor deficiencies and aberrant development of subtype-specific GABAergic interneurons caused by an unliganded thyroid hormone receptor alpha1. J. Neurosci. 28, 1904–1915.

Wallis, K., Dudazy, S., van Hogerlinden, M., Nordström, K., Mittag, J., and Vennström, B. (2010). The thyroid hormone receptor alpha1 protein is expressed in embryonic postmitotic neurons and persists in most adult neurons. Mol. Endocrinol. 24, 1904–1916.

Wells, S., and Murphy, D. (2003). Transgenic studies on the regulation of the anterior pituitary gland function by the hypothalamus. Front Neuroendocrinol. 24, 11–26.

Wémeau, J.L., Pigeyre, M., Proust-Lemoine, E., d'Herbomez, M., Gottrand, F., Jansen, J., Visser, T.J., and Ladsous, M. (2008). Beneficial effects of propylthiouracil plus L-thyroxine treatment in a patient with a mutation in MCT8. J. Clin. Endocrinol. Metab. 93, 2084–2088.

Wikström. L., Johansson, C., Salto, C., Campos, B.A., Forrest, D., Thoren, P., and Vennström, B. (1998). Abnormal heart rate and body temperature in mice lacking thyroid hormone receptor alpha1. EMBO J. 17, 455-461.

Williams, G.R. (2000). Cloning and characterization of two novel thyroid hormone receptor beta isoforms. Mol. Cell. Biol. 20, 8329–8342.

Wirth, E.K., Roth, S., Blechschmidt, C., Hölter, S.M., Becker, L., Racz, I., Zimmer, A., Klopstock, T., Gailus-Durner, V., Fuchs, H., et al. (2009). Neuronal 3',3,5-triiodothyronine (T3) uptake and behavioral phenotype of mice deficient in Mct8, the neuronal T3 transporter mutated in Allan-Herndon-Dudley syndrome. J. Neurosci. 29, 9439–9449.

Wu, D.B., Chen, E.Q., Bai, L., and Tang, H. (2017). Propylthiouracil-induced liver failure and artificial liver support systems: a case report and review of the literature. Ther. Clin. Risk Manag. 11, 65-68.

Xu, H., Yang, Y., Tang, X., Zhao, M., Liang, F., Xu, P., Hou, B., Xing, Y., Bao, X., and Fan, X. (2013). Bergmann glia function in granule cell migration during cerebellum development. Mol. Neurobiol. 47, 833–844.

Xu, X., Roby, K.D., and Callaway, E.M. (2010). Immunochemical characterization of inhibitory mouse cortical neurons: three chemically distinct classes of inhibitory cells. J. Comp. Neurol. 518, 389-404.

Yamanaka, H., Yanagawa, Y., and Obata, K. (2004). Development of stellate and basket cells and their apoptosis in mouse cerebellar cortex. Neurosci. Res. 50, 13-22.

Yen, A., Norman, A.W., and Varvayanis, S. (2001). Nongenomic vitamin D3 analogs activating ERK2 in HL-60 cells show that retinoic acid-induced differentiation and cell cycle arrest require early concurrent MAPK and RAR and RXR activation. In Vitro Cell. Dev. Biol. Anim.

37, 93–99.

Yoshihara, H.A., Apriletti, J.W., Baxter, J.D., and Scanlan, T.S. (2003). Structural determinants of selective thyromimetics. J. Med. Chem. 3, 3152-3161.

Zada, D., Tovin, A., Lerer-Goldshtein, T., Vatine, G.D., and Appelbaum, L. (2014). Altered behavioral performance and live imaging of circuit-specific neural deficiencies in a zebrafish model for psychomotor retardation. PLoS Genet. 10, 9.

Zada, D., Tovin, A., Lerer-Goldshtein, T., and Appelbaum, L. (2016). Pharmacological treatment and BBB-targeted genetic therapy for MCT8-dependent hypomyelination in zebrafish. Dis. Model Mech. 9, 1339–1348.

Zada, D., Blitz, E., and Appelbaum, L. (2017). Zebrafish - An emerging model to explore thyroid hormone transporters and psychomotor retardation. Mol. Cell Endocrinol. 25, 53-58.

Zondek, H., and Leszynsky, H.E. (1956). [Extrathyroidal Basedow-like disease]. Schweiz Med. Wochenschr. 86, 308–311.

Zung, A., Visser, T.J., Uitterlinden, A.G., Rivadeneira, F., and Friesema, E.C.H. (2011). A child with a deletion in the monocarboxylate transporter 8 gene: 7-year follow-up and effects of thyroid hormone treatment. Eur. J. Endocrinol. 165, 823–830.

Acknowledgements

Here, I would like to take the opportunity to express my gratitude to some people without whom this dissertation would not have been possible.

First of all, I would like to express the deepest appreciation to my Prof. Dr. Heike Heuer, who let me be part of her research. I thank her for a continually and convincingly guidance and a persistent help by giving constructive suggestions and criticism during the planning and development of this project. Besides her tremendous academic support, I would also like to thank her for being my mentor, for rising my passion for scientific work by showing me the relevance of our profession.

In addition, I want to thank Prof. Dr. Christine Rose, Prof. Dr. Ellen Fritsche and Prof. Dr. Zeier for their support during my Ph.D. period and for being part of the thesis committee.

I also wish to acknowledge the help provided by Prof. Dr. Dagmar Führer-Sakel and the whole Department of Endocrinology for their warm welcome at the University Hospital in Essen. They made our movement much easier!

Further, I deeply want to thank Dr. Boyka Markova, Dr. Steffen Mayerl and Markus Korkowski for their patient support in our laboratory, for an inspiring collaboration and for helping me targeting technical challenges and learning new methods with their great ideas and vast experience.

I am also grateful to Dr. Jiesi Chen, Dr. Sivaraj Sundaram and Dr. Denica Doycheva for their pleasant company. It was great sharing laboratory with them not just as colleagues but also as amazing friends. Thanks for that great working atmosphere!

Moreover, I would like to thank the Sherman Foundation for their great and meaningful financial and ideological support.

I would like to offer my special thanks to my friends for letting me rely on them in any situation, for deliberating problems and findings in research but also happily talking about anything else.

I am grateful to Dr. Alexander Hof, who has provided me through moral and emotional support in my life.

Finally, I wish to thank my family whose love and guidance are with me in whatever I pursue and for their unbelievable support and encouragement throughout my whole study, sharing moments of both happiness and sadness.

Thank you all for the encouragement!

Declaration of honour

I hereby declare that I have completed the present work and written the thesis independently. Only original data as well as independently created graphics were used for the preparation of this thesis and were never subjected to manipulation in content. I have clearly indicated the presence of all materials I have cited from other sources. In the selection and use of materials, as well as preparation of the thesis, I have received support from my mentor, Prof. Dr. Heike Heuer. I did not seek for any other improper help from third parties. Furthermore, this thesis has not previously been published in the same or similar form in Germany or abroad, nor has it been submitted to any other institution as an examination paper for a state or other scientific examination. Finally, I am aware of the current doctoral regulations at the Faculty for Mathematics and Natural Sciences of the Heinrich Heine University of Duesseldorf.

Düsseldorf, 11.03.2020

Eva Salveridou

Curriculum Vitae

Personal data

Name:	Eva Salveridou
Nationality:	German/ Greek
Birth date:	July 27, 1990
Birth place:	Mönchengladbach, Germany

Education

Since Jan 2018:	PhD student in the group of Prof. Heuer at the Department of Endocrinology, Diabetes and Metabolism at the University Hospital, Essen
Jan 2016 - Dec 2017:	PhD student in the group of Prof. Heuer at the Leibniz Institute for Environmental Research (IUF), Düsseldorf
Jan 2016 - Oct 2018:	Master of Science in Psychology Institute for Healthpsychology, Fernuniversität Hagen Thesis title: Alzheimer´s disease and possible coping strategies for family members, grade 2.3
Feb 2014 – Jul 2015:	Master of Science in Neurobiology at the Heinrich Heine University Master thesis at the Leibniz Institute for Environmental Research (IUF), Ventura Laboratory Thesis title: Mitochondrial stress induces hypoxic-like preconditioning in neurons, grade 1.0
Apr 2011 – Dec 2015:	Bachelor of Science in Psychology Institute for Healthpsychology, Fernuniversität Hagen Thesis title: The Effect of working memory workout on intelligence, grade 1.3

Oct 2010 – Sep 2013: Bachelor of Science in Biology at the Heinrich Heine University
Bachelor thesis at the Institute for Neurobiology
Thesis title: Serotonin and Depression, grade 1.3

Awards

2018 Best Young Investigator´s Oral Presentation at the 33rd Workshop on Experimental Thyroid Research (Berlin, Germany).

2018 The ETA Jack Robbins Prize for the best presentation in the field of thyroid hormone transport at the 41st Annual European Thyroid Association (ETA) Meeting (Newcastle, England).

2019 Best poster presentation at the 18th day of research (Tag der Forschung) of the medical faculty of the university Duisburg-Essen (Germany).

Publications

1. Postnatal Triac treatment prevents neurodevelopmental and locomotor impairments in Mct8/Oatp1c1 deficient mice.

Jiesi Chen, **Eva Salveridou**, Steffen Mayerl, Lutz Liebmann, Sivaraj Mohana Sundaram, Denica Doycheva, Christian Hübner and Heike Heuer

Submitted

2. Tissue-specific function of thyroid hormone transporters: new insights from mouse model.

Eva Salveridou, Steffen Mayerl, Sivaraj Mohana Sundaram, Boyka Markova, Heike Heuer

Experimental and Clinical Endocrinology & Diabetes (2019).

3. Thyromimetic potential of novel thyroid hormone analogs in mouse primary neurons.

Eva Salveridou, Meredith D. Hartley, Thomas S. Scanlan, Jiesi Chen, Boyka Markova, Dagmar Führer, Heike Heuer

In preparation

4. Thyromimetic potential of sobetirome compounds in thyroid hormone transporter-deficient mice.

Eva Salveridou, Meredith D. Hartley, Thomas S. Scanlan, Jiesi Chen, Boyka Markova, Dagmar Führer, Heike Heuer

In preparation

5. Hypoxia-mimetic interventions confer protection against age- and stress-induced damage across species through reduced fatty acids accumulation.

Alfonso Schiavi, Alessandra Runci, **Eva Salveridou,** Natascia Ventura

In preparation

Oral presentations

Salveridou E, Thyroid hormone analogs for the treatment of patients with MCT8 Mutations. 33rd Workshop on Experimental Thyroid Research (AESF), Berlin, January 2018.

Salveridou E, Role of the murine TH transporters Mct8 and Oatp1c1 in skeletal muscle, cardiovascular and metabolic system. 6th Annual Meeting SPP1629 Thyroid Trans Act, Berlin, June 2018.

Salveridou E, Defining the cell-specific function of Mct8 in mice. 22nd Annual symposium of the Dutch Thyroid Research Foundation (RTH), Doorn, September 2018.

Salveridou E, Thyromimetic potential of novel TH analogs in mouse primary neurons and in TH transporter deficient mice. 41st Annual European Thyroid Association (ETA) Meeting. Newcastle, September 2018.

Salveridou E, Thyromimetic potential of novel TH analogs in mouse primary neurons and in TH transporter deficient mice. 48. Jahrestagung der Sektion Schilddrüse der Deutschen Gesellschaft für Endokrinologie, Essen, November 2018.

Salveridou E, Action of novel TH analogs in primary neurons and in thyroid hormone transporter deficient mice. 24. Jahrestagung Nordrhein-Westfälischer Gesellschaft für Endokrinologie & Diabetologie. Dortmund, February 2019.

Salveridou E, Action of novel TH analogs in primary neurons and in thyroid hormone transporter deficient mice. 62. Deutscher Kongress für Endokrinologie (DGE), Göttingen, March 2019.

Salveridou E, Effects of TH analogs on primary neurons and in thyroid hormone transporter deficient mice. 21st Annual Conference of the Young Active Research in Endocrinology (YARE) Initiative, Essen, October 2019.

Poster presentations

Salveridou E, Chen J, Korkowski M, Heuer H. Testing of novel thyroid hormone analogs in thyroid hormone transporter deficient mice. ESE Summer School on Endocrinology, Bregenz, July 2016.

Salveridou E, Hartley D. M, Scanlan T. S, Führer D, Heuer H. Effects of TH analogs on primary neurons and in thyroid hormone transporter deficient mice. Tag der Forschung, Essen, November 2019.

Salveridou E, Hartley D. M, Scanlan T. S, Führer D, Heuer H. Thyromimetic effects of sobetirome compounds *in vitro* and *in vivo*. 14th International Workshop on Resistance to Thyroid Hormone & Thyroid Hormone Action, Monterey, California, March 2020.