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Brain, mood and cognition in hypothyroidism

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Referat:
Adult-onset hypothyroidism leads to mood and cognitive impairment (Canaris et al., 1997; Joffe et al., 2012). Residual symptoms may even persist in patients with biochemically adequate treatment (Saravanan et al., 2002). Reasons discussed for residual symptoms include firstly independent effects of autoimmune processes on the brain (Grabe et al., 2005), secondly brain hypothyroidism in spite of normal serum hormone levels (Panicker et al., 2009a), thirdly comorbidities leading to a selection bias in seeking health care (Kong et al., 2002) and fourthly reactive mental processes to the awareness of having a chronic disease (Ladenson, 2002). Disentangling these possible causes has important implications for treatment strategies. The present study wants to contribute to the discussion by studying the target organ of interest, the brain, and measuring levels of autoimmunity. Animal studies have indicated that adult-onset hypothyroidism leads to impaired memory, anxiety and depression associated with alterations in hippocampal and amygdalar neuronal plasticity (Alzoubi et al., 2009; Montero-Pedrazuela et al., 2006, 2011). Magnetic resonance imaging (MRI) is a powerful non-invasive tool to investigate the effects of hormone action on structure and function of the brain in living patients (Brabant et al., 2011; Pilhatsch et al., 2011) and will be used to study neural correlates of hypothyroidism in the current research project. First data from invasive human brain imaging studies have shown changes in brain glucose metabolism and cerebral blood flow in hypothyroidism, but as of yet studies are inconsistent concerning reversibility and localisation (Bauer et al., 2009; Constant et al., 2001; Krausz et al., 2004).
As a prerequisite for the MRI study, we successfully translated and validated a set of questionnaires (McMillan et al., 2006; 2008) to measure symptoms, quality of life and treatment satisfaction in hypothyroidism (publication 1). In the main study, we found subclinically reduced mood in long-term adequately treated patients, but the mood alterations were not associated with alterations in depression-related brain networks. We identified thyroid autoimmunity and treatment duration as factors of neural alterations in long-term treated hypothyroidism. In the control group comparison we did not find structural and functional brain alterations (publication 2). More evidence is needed on neural alterations in hypothyroidism, ideally from larger population-based samples. Nonetheless, in long-term treated patients presenting with residual symptoms, alternative causes such as comorbidities and reactive mental processes to the awareness of having a chronic disease should be considered.

List of Abbreviations

BOLD	Blood oxygen level dependent
CBF	Cerebral blood flow
fMRI	Functional magnetic resonance imaging
T3	Triiodothyronine
T4	Tetraiodothyronine
LTP	Long-term potentiation
MR	Magnetic resonance
MRI	Magnetic resonance imaging
PET	Positron emission tomography
PROMs	Patient-reported outcome measures
ROI	Region of interest
SPECT	Single photon emission computed tomography
ThyDQoL	Thyroid-dependent quality of life questionnaire
ThySRQ	Underactive thyroid symptom rating questionnaire
ThyTSQ	Thyroid treatment satisfaction questionnaire
TPO-ab	Thyroid peroxidase antibodies
TSH	Thyroid stimulating hormone
VBM	Voxel-based morphometry

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I Introduction

Hypothyroidism during foetal brain development leads to severe and irreversible mental retardation (Führer et al., 2014), known as cretinism. It is mainly caused by severe iodine deficiency. The prevalence for this condition has been reduced by a highly successful public health program to eliminate iodine deficiency disorders by introducing iodised salt to affected populations. It has been launched by the World Health Organisation in 1990 and until 2000 the access of households to iodised salt increased from 20-30% to 70% (Hetzel, 2005).

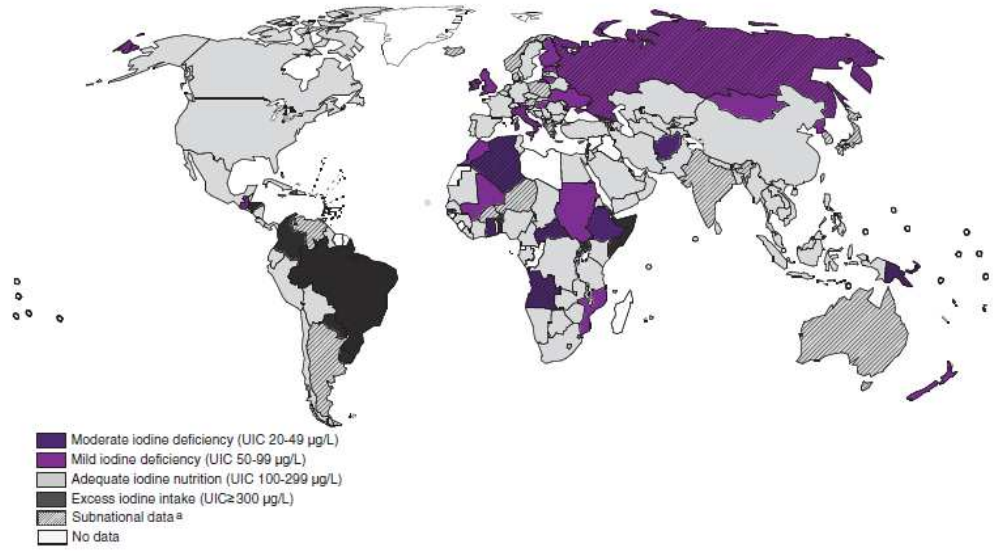

Figure 1: National iodine status in 2013 showing urinary iodine concentrations (UIC) of school-aged children. Subnational data means that data does not cover the whole country and iodine intake may vary across subregions. Figure reprinted with permission from Pearce et al., 2013.

However, inadequate levels of iodine intake, including iodine excess, are still present in many countries (Pearce et al., 2013; see Fig. 1). Germany was still listed as a iodine deficient country in 2004 (Delange et al., 2004), but recent studies report adequate iodine levels (Meisinger et al. 2012; Pearce et al., 2013; Völzke et al.,

2012). Important to note, in contrast to the clear association between severe iodine deficiency and cretinism, but also thyroid enlargement (goitre), there is a more complex relationship between mild iodine deficiency or excess iodine intake and prevalence rates for thyroid diseases with onset in adulthood (Laurberg et al., 2010). Autoimmune thyroiditis, or Hashimoto's disease, is the most common cause for adult-onset hypothyroidism in Germany (Gärtner et al., 2002; see chapters II.1.2 and II.1.3). It occurs more often in goitre patients and incidence rates are expected to decrease with increasing iodine availability (Laurberg et al., 2010). However, it could be shown that sudden rises in iodine uptake in previously iodine deficient populations, as happens during the implementation phase of iodination programs, increase the prevalence for autoimmune thyroiditis (Kahaly et al., 1998; Pedersen et al., 2011) and associated hypothyroidism (Bjergved et al., 2012; Meisinger et al., 2012). Therefore, we can currently even expect a transient increase in adult-onset autoimmune hypothyroidism in Germany for those born before 1990 or even 2004, and this patient group will be addressed in the current research project. Prevalence estimates for hypothyroidism or subclinical hypothyroidism are 5% for the whole population, with higher rates in women (8%) than men (3%) and an increase with age up to 21% in females and 16% of males over the age of 74 (Roberts and Ladenson, 2004; Vanderpump, 2011).

Although the neurocognitive effects of adult-onset hypothyroidism are not as deleterious as seen during foetal development, impairment of mood and cognition has reliably been reported for severe, partly also for mild adult-onset hypothyroidism (Allolio and Schulte, 1996; Hetzel, 2005; Roberts and Ladenson, 2004). Residual symptoms may even persist in patients with biochemically adequate treatment (Saravanan et al., 2002; Wekking et al., 2005; see chapters II.1.4 and II.1.5).

Reasons discussed for residual symptoms include firstly independent effects of autoimmune processes on the brain (Grabe et al., 2005), secondly brain hypothyroidism in spite of normal serum hormone levels (Panicker et al., 2009a), thirdly comorbidities leading to a higher chance of having thyroid hormone values tested (Kong et al., 2002) and fourthly reactive mental processes to the awareness of having a chronic disease (Ladenson, 2002). Disentangling these possible causes has important implications for treatment strategies. The present study wants to contribute to the discussion by studying the target organ of interest, the brain, and individual levels of autoimmunity. Studies of the brain in hypothyroid rodents have shown impaired cell signalling in hippocampus and amygdala related to memory and depression (Alzoubi et al., 2009; Montero-Pedrazuela et al., 2006, 2011; see chapter II.3.1). However, little is known about neural correlates of hypothyroidism in humans. Magnetic resonance imaging (MRI, see chapter II.2) is a powerful non-invasive tool to investigate the effects of hormone action on structure and function of the brain in living patients (Brabant et al., 2011; Pilhatsch et al., 2011) and will be used to study neural correlates of hypothyroidism here. First invasive human brain imaging studies have shown changes in brain glucose metabolism and cerebral blood flow in hypothyroidism, but as of yet studies are inconsistent concerning reversibility and localisation (Bauer et al., 2009; Constant et al., 2001; Krausz et al., 2004; see chapter II.3.2).

We are interested both in initial treatment effects of levothyroxine to reestablish euthyroidism as well as in long-term treatment effects to investigate possible causes for residual symptoms. In order to study independent effects of the autoimmune process, we measure thyroid peroxidase antibodies (TPO-ab) in addition to thyroid hormone values.

In the following chapter I will summarise the current state of the literature in this interdisciplinary research field. This will include sections on the thyroid hormone system, (autoimmune) hypothyroidism, related mood and cognitive impairment, the MRI methods used, and animal as well as human brain imaging studies. Consequentially, I will derive the rational for the experimental work (chapter II). Our experimental work resulted in two publications that form the present cumulative dissertation (chapter III). I will close with a general discussion and some proposals for future investigations (chapter IV).

II Theoretical Background

1 The thyroid and its dysfunction

1.1 The thyroid hormone system

The thyroid is a butterfly-shaped endocrine gland situated below the larynx (Ehlert and von Känel, 2011). It produces the hormone triiodothyronine (T3) and the pro-hormone tetraiodothyronine (T4) and releases them into the blood stream, where they are mainly bound to transport proteins. The small portion of unbound (pro-) hormones are called free T3 (fT3) and free T4 (fT4), respectively. At target cells, including neurons, fT3 binds to thyroid hormone receptors in the cell membrane and thus influences gene transcription in the cell (Janssen et al., 2001). Furthermore, it can lead to an increase in glucose uptake into the cells leading to general metabolic enhancement (Roberts and Ladenson, 2004). fT4 itself is not active, it needs deiodinisation by the enzymes deiodinase 1 or 2 (d1/d2) to fT3 at the target cell to become effective. 80% of fT3 is produced by this production on demand (Dayan and Panicker, 2009). The thyroid hormone system is feedback-regulated via the hypothalamus and the pituitary gland that releases thyroid stimulating hormone (TSH) to the thyroid gland in order to increase T3 and T4 release if hormone levels are too low and vice versa (see Fig. 2 for a schematic view). TSH is the most sensitive indicator for a dysfunction of the thyroid hormone system (Boelart and Franklyn, 2005; Ehlert and von Känel, 2011). Functional disorders of the thyroid are classified according to three serum parameters: TSH, fT4 and fT3. High TSH levels (>4mU/l) indicate hypothyroidism, whereas low TSH levels (<0,4mU/l) are defined as hyperthyroidism (Pfannenstiel et al., 1999). As long as TSH is altered in isolation, the condition is called subclinical. If in addition fT4 or fT3 are outside reference levels,

the conditions are called overt. In the present work I focus on the condition of hypothyroidism and will now focus on this part of the thyroid hormone continuum.

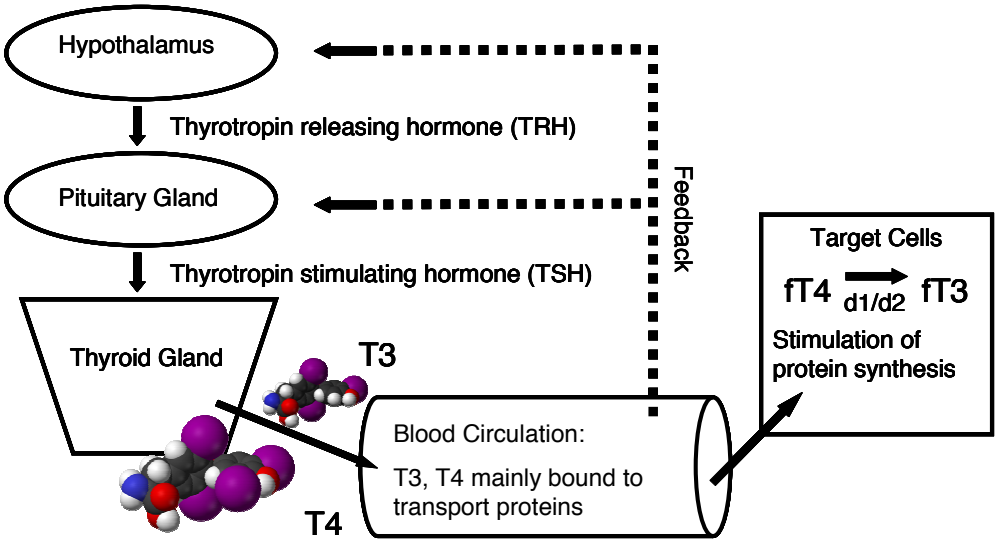

Figure 2: The thyroid hormone cycle. Own figure inspired by figure 1.3 in Ehlert and von Känel, 2011 and figure 1 in Dayan and Panicker, 2009.

1.2 Hypothyroidism

The main clinical symptoms of hypothyroidism are weight gain, cold intolerance, tiredness, obstipation, bradycardia, hair loss, depression and cognitive deficits (Allolio and Schulte, 1996; Roberts and Ladenson, 2004). Hypothyroidism is routinely treated with synthetic thyroxine, called levothyroxine, restoring hormone levels to the normal range. It is still under discussion whether some patients may benefit from additional replacement with fT3, mimicking the exact output of the healthy thyroid that produces small amounts of fT3 in addition to fT4 (Roberts and Ladenson, 2004).

The most frequent cause of adult-onset hypothyroidism is autoimmune thyroiditis due to TPO-ab (Hashimoto's thyroiditis), affecting 1-2% of the population (Gärtner et al.,

2002). The second most common cause is medical intervention, including thyroidectomy or radioiodine treatment for thyroid cancer or hyperthyroidism. This patient group experiences abrupt hypothyroidism after the intervention and is then timely treated with levothyroxine. Cancer patients receive supraphysiologically high doses to suppress TSH for preventive reasons. Other rare causes are the so-called secondary forms of hypothyroidism due to deficits within the thyroid feedback cycle, which are TSH or thyrotropin releasing hormone (TRH) deficiency (Pfannenstiel et al., 1999). For a control group study on hypothyroidism the first group of patients with Hashimoto's thyroiditis is best suited for several reasons. Firstly, prevalence is highest, secondly treatment aims at a euthyroid status that is comparable to normal thyroid function, thirdly no iatrogenic intervention has taken place that could possibly influence results by the intervention itself or the initial disease. Finally, independent effects of autoimmune thyroid activity on mood are discussed in the literature on hypothyroidism and can be studied in this patient group (Gärtner et al., 2002; Grabe et al., 2005; Ott et al., 2011).

1.3 Autoimmune thyroiditis (Hashimoto's disease)

The cause of the most common autoimmune disease (Ehlert and von Känel, 2011) was unclear until the concept of autoimmunity became known in the 1950's and it was not until the 1980's that thyroid peroxidase was identified as the main target of the immune system in patients with Hashimoto's thyroiditis (Caturegli et al., 2013). TPO-ab are found in 90-95% of patients (Janssen et al., 2001; Roberts and Ladenson, 2004). The presence of TPO-ab highly increases the likelihood of developing hypothyroidism (Vanderpump and Tunbridge, 2002), wherefore the

presence of antibodies is used for initial treatment decisions (Allolio and Schulte, 1996; Prummel and Wiersinga, 2005). Hashimoto's thyroiditis is equally treated by levothyroxine, as causal cures targeting the autoimmune process are still scarce. However, first attempts using selenium have been made (Ott et al., 2011). It has been argued that independent of the symptoms caused by the hypothyroidism, the autoimmune process may exhibit an independent effect on patients' mood and cognition (Gärtner et al., 2002; Grabe et al., 2005; Leyhe et al., 2008; Ott et al., 2011). Examining the independent effect of TPO-ab on mood, cognition and brain is therefore of special interest to the current study.

1.4 Mood in hypothyroidism

Reduced perceived health and more hypothyroidism-related symptoms were found in overtly hypothyroid patients (Canaris et al., 1997; Schraml and Beason-Held, 2010). Concerning subclinical hypothyroidism, the literature is less decided (Joffe et al., 2012). On the one hand, no heightened depressivity or anxiety was found in some studies (Bono et al., 2004). However, levothyroxine removal in long-term treated patients leading to subclinical hypothyroidism revealed a reduction in perceived health and well-being (Samuels et al., 2007).

Concerning the initial treatment effect of levothyroxine replacement therapy, some studies found an improvement (Bono et al., 2004; Correia et al., 2009; Miller et al., 2006), although not all report complete normalisation. Other placebo-controlled treatment studies on mildly hypothyroid subjects found no changes beyond the placebo effect (Jorde et al., 2006; Kong et al., 2002). Most studies on long-term treated hypothyroidism found impaired perceived health status and increased mental

strain (Samuels et al., 2007; Saravanan et al., 2002; Wekking et al., 2005) and an increased prevalence of depression and anxiety (Panicker et al., 2009b).

Concerning autoimmune thyroid disease, reduced perceived health was found in euthyroid Hashimoto's disease (Bianchi et al., 2004; Ott et al., 2011). In the general population, autoimmune thyroid disease was associated with increased anxiety, physical symptoms and depression (Grabe et al., 2005; Pop et al., 1998). One study showed that TPO-ab activity can be reduced by selenium supplementation alongside subjective health improvement (Gärtner et al., 2002), suggesting an interesting new treatment approach.

In conclusion, whereas mood alterations are robustly reported in overt untreated hypothyroidism, the literature is less coherent concerning untreated subclinical hypothyroidism and levothyroxine-treated conditions. It is still an open issue where residual alterations despite adequate treatment result from. Explanations discussed are insufficient normalisation of thyroid hormone levels at target tissues such as the brain despite normal serum hormone levels (Panicker et al., 2009a), independent effects of thyroid autoimmunity by TPO-ab (Ott et al., 2011), selection bias in seeking health care (Kong et al., 2002) and reactive mental processes to the awareness of having a chronic disease (Ladenson, 2002). The current study wants to add to the discussion by investigating neural correlates of mood during treatment initiation and in long-term treated hypothyroidism of autoimmune origin.

Patient-reported outcome measures (PROMs) used to study mood in hypothyroidism so far have mainly been generic instruments that are applicable to several diseases such as the Short-Form 36 (Bullinger and Kirchberger, 1998), a questionnaire on self-reported mental and physical health, or questionnaires measuring depressive symptoms (Hamilton depression scale, Baumann, 1976). They are therefore widely-

used and well-suited for comparisons between studies, but instruments specific for a certain disease have been shown to be more sensitive to change (Eurich et al., 2006; de Vries et al., 2005). For the assessment of hypothyroidism-specific symptoms, homemade symptom lists have been used so far (Jorde et al., 2006; Ott et al., 2011; Saravanan et al., 2002), leading to little comparability across studies. Hence, we wanted to include validated disease-specific instruments in addition to well-known generic instruments and as none were available in German, we translated and validated a set of English questionnaires in cooperation with the original author team (McMillan et al., 2006; 2008). This important methodological prerequisite forms part of the first publication of this dissertation.

1.5 Cognition in hypothyroidism

The cognitive domains most discussed in hypothyroidism are memory, working memory, attention and psychomotor speed. Studies are highly heterogeneous as to the severity, treatment status, cause of disease, age range and study population and have produced equally heterogeneous results.

Verbal or spatial memory deficits (Baldini et al., 1997; Burmeister et al., 2001; Correia et al., 2009; Miller et al., 2006), as well as working memory impairment (Schraml et al., 2011) have been reported in untreated hypothyroidism. The same studies found no attention deficit (Burmeister et al., 2001; Miller et al., 2006) and other studies found neither memory nor attention to be impaired in untreated hypothyroidism (Bono et al., 2004; Jorde et al., 2006; Schraml et al., 2011). Interestingly, one study found significant subjective impairment in broad cognitive domains despite objective impairment in verbal memory only (Burmeister et al.,

2001). Small TSH changes within the normal range did not produce alterations in attention or working memory (Walsh et al., 2006), whereas levothyroxine removal in long-term treated patients leading to subclinical hypothyroidism led to a reduction in working memory ability (Samuels et al., 2007). Two reviews on the topic conclude at least for subclinical hypothyroidism that the inconsistent findings suggest at most rather subtle cognitive deficits (Joffe et al., 2012; Samuels et al., 2010).

Treatment initiation with levothyroxine led to a significant improvement in most studies (Baldini et al., 1997; Burmeister et al., 2001; Jaeschke et al., 1996; Miller et al., 2006), but not always normalised to control group levels (Correia et al., 2009) and was indistinguishable from placebo control groups in other studies (Jorde et al., 2006; Parle et al., 2010).

Studies on long-term treated hypothyroidism found impaired memory and attention (Wekking et al., 2005) and another study impaired working memory and motor learning (Samuels et al., 2007), whereas no cognitive impairment was found in other studies (Kramer et al., 2009). Studies on the role of autoimmunity on cognition in hypothyroidism are scarce. One study on long-term treated patients with Hashimoto's thyroiditis showed a very specific attention deficit (Leyhe et al., 2008).

In conclusion, the behavioural literature on cognition in hypothyroidism is indecisive, especially concerning subclinical and long-term treated hypothyroidism. A potentially independent role of autoimmunity on cognition as suggested for mood impairment (Gärtner et al., 2002; Grabe et al., 2005) has so far not been studied. We therefore find it warranted to include high-load cognitive testing in the present work to investigate potentially subtle cognitive impairment and to pay special attention to autoimmune involvement.

2 Magnetic resonance imaging

2.1 Physical basis

The magnetic resonance (MR) technique is based on an intrinsic property of (hydrogen) protons, namely their spin. It means that they contain a perpetual rotating charge that produces a measurable stable magnetic axis. If many protons of e.g. a human brain enter a strong magnetic field such as an MR scanner, the magnetic axes of some of these protons align with the magnetic field, leading to a measurable net magnetisation along the scanner magnet (Jäncke, 2005; Schneider and Fink, 2013). During MR measurement this magnetisation is excited by a high frequency electromagnetic wave causing the protons to turn their magnetic axis by the angle the wave is send out. This produces a measurable alternating voltage. After switching off the electromagnetic wave the system regains the stable magnetic field. Different processes reflect the decay of the induced transverse magnetisation, called T1-, T2- and T2*-relaxation (Logothetis, 2008). The process of excitation and relaxation is repeated many times to gather robust data. The differentiation of tissue types in the brain is possible because different characteristics of the tissues such as proton density or magnetic properties influence the relaxation times. Acquisition parameters, e.g. the repetition time between the excitations, can be adapted to optimise contrast between tissues of interest. In addition, the signal depends on the magnetic field strength, allowing for detailed spatial coding. In order to reconstruct a 3-dimensional image of the brain, a grid of unique magnetic field strengths is established by adding three magnetic gradients along the three spatial axes to the main magnetic field (Buxton, 2009; Weishaupt et al., 2009).

2.2 Structural and functional MRI

For analysing brain structure, the properties of the three main tissue types of the brain: grey matter, white matter and cerebrospinal fluid, and their influence on the relaxation times, are crucial. T1-relaxation times differentiate best between grey and white matter, wherefore T1-weighted contrast images are most often used for structural analyses. The probability of each voxel belonging to one of the three tissue types can be calculated by voxel-based morphometry (VBM, Ashburner and Friston, 2000).

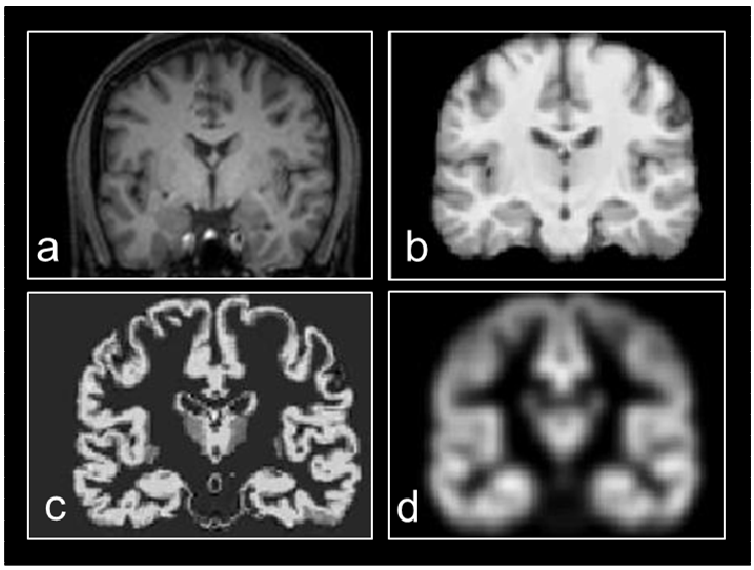

Figure 3: Own example dataset illustrating structural MRI preprocessing using voxel-based morphometry, (a) structural T1 image, (b) mean image after normalisation, (c) extracted grey matter tissue, (d) 8mm smoothed image.

The magnetisation-prepared rapid gradient echo sequence (MP-RAGE) is optimised to differentiate between grey matter (darker) and white matter (lighter), cerebrospinal fluid appears black (Schneider and Fink, 2013; see Fig. 3 a) and has therefore been used here. Raw structural images require preprocessing before use in group

analyses in order to ensure comparability between subjects for later voxel-wise comparison of grey matter probability values. Preprocessing includes normalisation to a standard brain, here the Montreal Neurological Institute (MNI) space as well as segmentation of the different tissue types and smoothing by a Gaussian Kernel (Ashburner and Friston, 2000; see Fig. 3). Details of acquisition parameters, data processing and group analyses are given in publication 2 (chapter III.1) and the respective supplemental material (chapter VII.1).

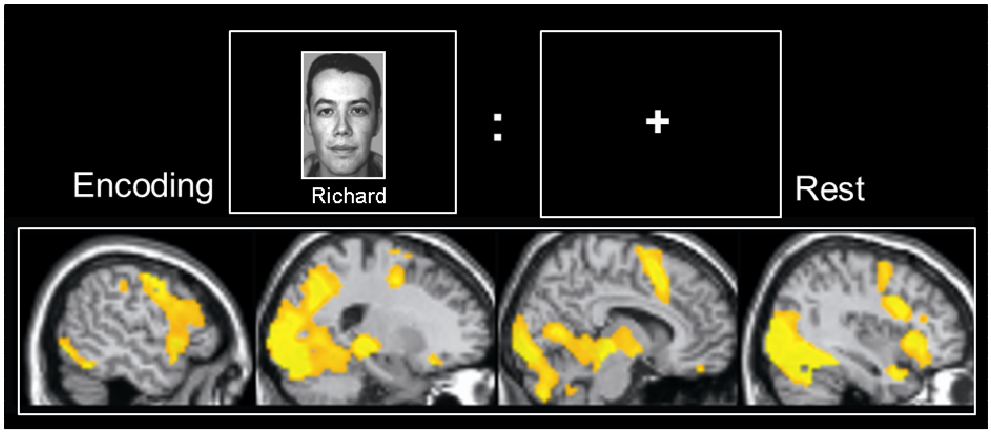

Figure 4: Illustration of the contrast method used in task-based fMRI. Results of a onesample t-test showing significantly higher BOLD responses to memory encoding vs. rest in healthy participants overlayed on a structural MRI scan. Modified from figures 1 and 3, Quinque et al., 2013.

Functional MRI (fMRI) exploits the so-called BOLD-effect, the blood oxygen level dependent effect. Neurally active brain areas expend more oxygen leading to a local increase in blood flow beyond actual oxygen requirement. As a result, there is a transient increase in oxyhaemoglobin and a reduction in deoxyhaemoglobin. The T2*-relaxation time is susceptible to magnetic properties of tissues and as oxyhaemoglobin and deoxyhaemoglobin differ in this respect, T2*-weighted images

reflect changes in blood-oxygen levels over time, providing an indirect measure of neural activity (Buckner and Logan, 2001; Logothetis, 2008).

In resting-state fMRI spontaneous BOLD signal changes over time in a resting participant are investigated allowing the analysis of neural connectivity between brain regions. This neural connectivity represents an important feature of the brain, namely its organisation in functional networks. It thus ideally supplements structural MRI methods (e.g. VBM) that study brain regions in isolation or task-based fMRI studies that are always confined to the specific domain studied (Biswal et al., 1995; Fox and Raichle, 2007). A large array of processing options has been developed to address several research questions (Margulies et al., 2010). In the case of a priori information about regions of interest (ROI), seed-based analysis is suitable. Seed-based resting-state fMRI looks into the correlation of the average BOLD time course of the ROI with the BOLD time course of all other voxels in the brain. The physiological rational behind this analysis is the idea that the amount of shared functionality is related to the synchrony of time courses during rest (Fox and Raichle, 2007). Bauer et al., (2009) provide region-specific data about brain areas related to altered glucose metabolism in hypothyroidism. Therefore, we were able to enter valid a priori seeds to investigate functional connectivity in hypothyroidism and used this informed approach in the current study, see publication 2 (chapter III.1) and the respective supplemental material (chapter VII.1).

3 Hypothyroidism and the brain

3.1 Animal studies

Animal studies on hypothyroidism suggest both behavioural deficits and correlated physiological pathomechanisms. Several studies found that hypothyroid rats are impaired in spatial learning, accompanied by a reduction in long-term potentiation (LTP) in the hippocampus, a neurophysiological correlate of memory (Alzoubi et al., 2009; Artis et al., 2011). Both memory impairment and LTP reduction were reversible with levothyroxine treatment (Alzoubi et al., 2009; see Fig. 5).

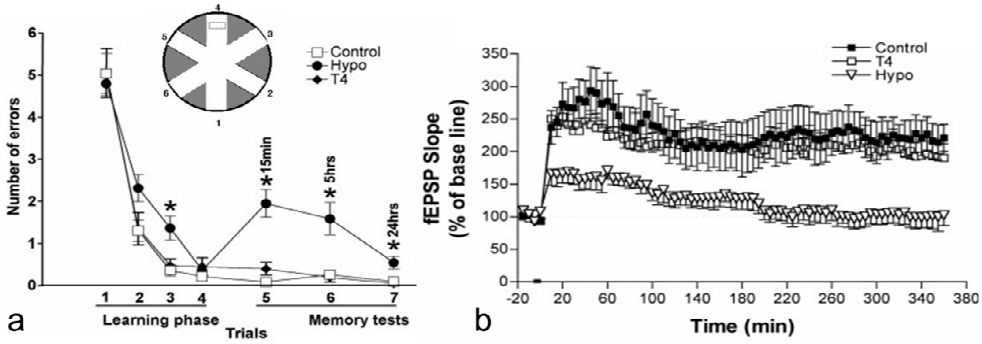

Figure 5: Hypothyroid rats show reversibly impaired learning and memory in a radial arm water maze (a) and reversibly reduced excitatory postsynaptic field potentials (fEPSP), a measure of long-term potentiation in the hippocampus (b). Modified with permission from Alzoubi et al., 2009.

In contrast, Fernández-Lamo et al., (2009) report impairment in associative learning and altered protein expression involved in LTP induction despite adequate treatment (see Fig. 6). Levothyroxine treatment prevented memory impairment and hippocampal cell death in an Alzheimer's disease mouse model (Fu et al., 2010). Further hippocampal alterations as known from Alzheimer's disease such as atrophy, inflammation (Chaalal et al., 2014) or amyloid-β peptide formation (Ghenimi et al.,

2010) have been found in hypothyroidism and linked to memory impairment. Moreover, the formation of new hippocampal neurons was found to be impaired because of reduced survival of neural cell progenitors (Ambrogini et al., 2005; Cortés et al., 2012; Desouza et al., 2005; Montero-Pedrazuela et al., 2006).

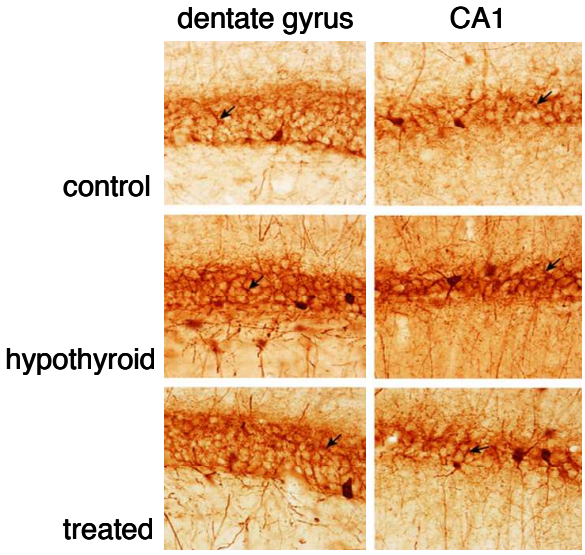

Figure 6: The expression of parvalbumin is increased in dentate gyrus and CA 1 regions of the hippocampus in thyroidectomised hypothyroid rats and adequately treated rats. Parvalbumin is a protein involved in LTP induction. Modified with permission from Fernández-Lamo et al., 2009.

The reported studies induced severe hypothyroidism in order to achieve proof of principle in experimental models either by thyroidectomy or by applying high doses of an anti-thyroid agent. A single study explicitly elicited only subclinical hypothyroidism by partial thyroidectomy and again showed memory impairment alongside alterations in the hippocampal signalling cascade (Ge et al., 2012). Studies may thus be generalised to subclinical hypothyroidism as often encountered in clinical practice, but more animal studies in mild hypothyroidism are needed.

In the affective domain, hypothyroidism led to enhanced fear conditioning coinciding with increased corticosterone release and a heightened number of corticosteroid receptors in the amygdala (Montero-Pedrazuela et al., 2011). A genetically caused form of brain hypothyroidism produced depressive and anxious behaviour that was successfully treatable with hormone replacement (Pilhatsch et al., 2010) and reduced survival of neural cell progenitors in the hippocampus could be associated with depressive behaviour (Montero-Pedrazuela et al., 2006).

Although research has focussed on the hippocampus and related memory impairment, other brain regions have been shown to be altered by hypothyroidism as well, e.g. the amygdala as described above (Montero-Pedrazuela et al., 2011). Moreover, T3 and T4 levels were undetectable or significantly reduced not only in hippocampus and amygdala, but also in parietooccipital cortex, limbic forebrain, hypothalamus, midbrain, cerebellum, medulla and frontal cortex (Broedel et al., 2003). Finally, levels of the neurotransmitters dopamine, norepinephrine and serotonin have recently been shown to be reduced in cerebral cortex, thalamus, midbrain, cerebellum and pons-medulla (Hassan et al., 2013).

We will include an associative memory fMRI task known to elicit hippocampal activity (Henke et al., 1997) in order to investigate hippocampal alterations in hypothyroidism as suggested by animal research. In addition, we will study structural and resting-state functional MRI of the whole brain in order to observe effects of the pathomechanisms reported for several brain regions.

3.2 Human brain imaging studies

Brain glucose metabolism and cerebral blood flow (CBF), measured by positron emission tomography (PET) and single photon emission computed tomography (SPECT), invasive brain imaging methods requiring the injection of radioactive tracers, have reliably shown changes in overt and subclinical hypothyroidism. Alterations proved reversible shortly after reestablishment of euthyroidism in some studies (Constant et al., 2001; Bauer et al., 2009; Schraml and Beason-Held, 2010), while others showed persistent alterations (Krausz et al., 2004; Nagamachi et al., 2004).

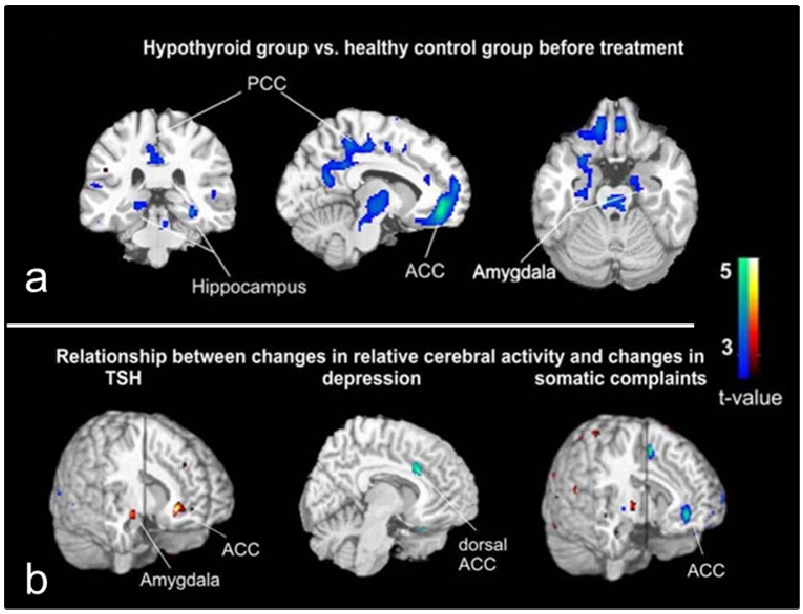

Figure 7: Group differences in glucose metabolism before treatment (a) and correlation of treatment-related changes in metabolism with changes in clinical parameters (b). Figures modified with permission from Bauer et al., 2009.

In terms of localisation, diffuse (Constant et al., 2001) or regionally specific alterations have been reported, although reports include areas across all four brain

lobules (Bauer et al., 2009; Krausz et al., 2004; Nagamachi et al., 2004; Schraml and Beason-Held, 2010). Direct associations between neural alterations and clinical parameters such as thyroid hormone status, mood and cognition are far less studied. Schraml and Beason-Held (2010) showed an association between TSH and CBF in occipital cortex, psychomotor speed and precentral gyrus as well as depressivity and frontal and thalamic areas during overt hypothyroidism. Bauer et al. (2009) showed that the restoration of glucose metabolism was correlated with the normalisation of TSH, symptom load and mood in anterior cingulate cortex and amygdala (see Fig. 7).

In addition to invasive brain imaging methods, a series of working memory task-based fMRI studies in adult-onset hypothyroidism has been performed before and shortly after treatment initiation. Reversible alterations in attentional load effects in frontoparietal areas have been found (He et al., 2011; Yin et al., 2013; Zhu et al., 2006). Task-based studies on other cognitive functions discussed in hypothyroidism such as memory are lacking. No brain imaging study is known to us on associative memory in adult-onset hypothyroidism in spite of extensive animal literature on hippocampal involvement in hypothyroidism (see section 3.1). However, there is evidence from teenagers with congenital hypothyroidism that hippocampal activity is altered during an associative memory task despite unimpaired behavioural performance (Wheeler et al., 2011).

Brain imaging studies explicitly on patients with Hashimoto's thyroiditis have so far been confined to SPECT analyses and have reliably shown diffuse hypoperfusion in different samples including unmedicated euthyroidism (Piga et al., 2004), untreated hypothyroidism (Kaya et al., 2007) and euthyroidism under adequate treatment (Zettinig et al., 2003). In the adequately treated patients hypoperfusion was not correlated with current TSH, TPO-ab levels, anxiety or depression, but only to the

duration of the disease (Zettinig et al., 2003). These SPECT data suggest brain alterations specific to Hashimoto's disease, but data on other brain imaging modalities are lacking and will be investigated here.

Two recent structural MRI studies in severely hypothyroid patients, published concurrently with our own, report either widespread alterations in grey and white matter (Singh et al., 2013) or a decrease in right hippocampal volume (Cooke et al., 2014). However, both studies neither report behavioural measures nor treatment effects, so that we do not know whether the reported findings are reversible and related to symptoms. We will try to answer these questions here.

Brain imaging data on long-term treated hypothyroidism are scarce, but may well differ from short-term treatment effects as suggested by persistent hypoperfusion shown in long-term adequately treated Hashimoto's disease patients (Zettinig et al., 2003). A recent VBM study included longer-term treated Hashimoto's thyroiditis patients, but reports only a very specific correlation between a small region of interest (left inferior frontal gyrus) and performance in a single task (Leyhe et al., 2013). More data is thus needed on brain structure and function in long-term treated patients with Hashimoto's thyroiditis. We will include a respective patient group here.

Resting-state fMRI allows the study of functional connectivity, extending the analysis of neural activity in isolated regions to the physiologically more valid assessment of neural networks spanning several brain regions (Fox and Raichle, 2007). Connectivity networks have been shown to be altered in related diseases such as Alzheimer's disease (Greicius et al., 2004). Therefore, we want to introduce it to the study of hypothyroidism here.

4 Rational of the experimental work

From the current state of the literature as summarised above, we derived the need for the following experimental work:

The lack of appropriate disease-specific PROMs was identified and overcome by translating and validating a set of existing English questionnaires. Furthermore, we identified both newly diagnosed patients as well as longer-term adequately treated patients as study groups of interest for the main MRI study, because both initial and residual cognitive and affective symptoms have been reported and their neural correlates may help us to understand their cause. Moreover, we saw the need for paying special attention to independent effects of autoimmunity and thus included patients with autoimmune thyroiditis and measured TPO-ab levels in serum in addition to thyroid hormone levels.

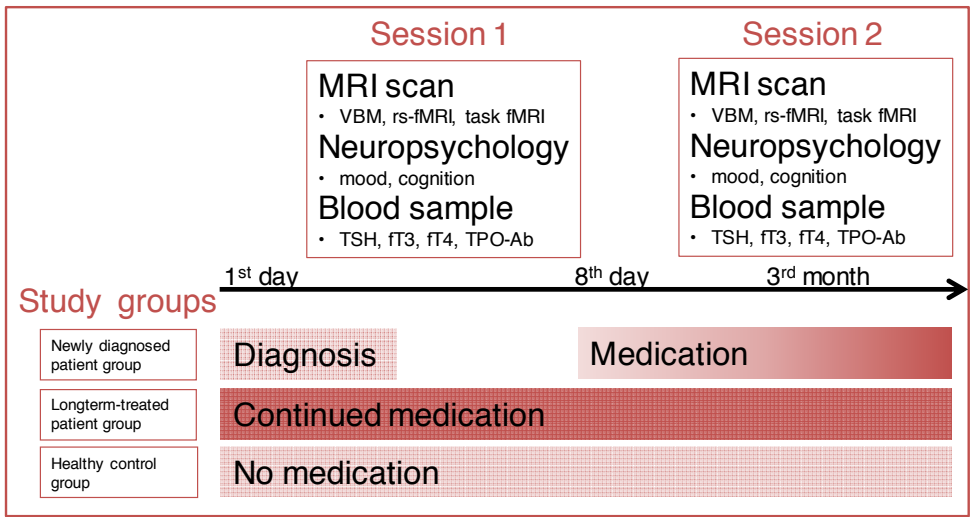

Figure 8: Schematic view of the study design.

Finally, as to the level of analysis and hence the selection of the employed methods, we wanted to bridge a gap between the mainly behavioural literature on human patients and the invasive animal literature on pathomechanisms at a cellular level. We therefore applied structural and functional MRI as well as thorough neuropsychological testing of mood and cognition within a single study. Fig. 8 provides a schematic description of the study design.

We hypothesise that untreated and possibly also long-term treated patients show mood and cognitive impairment that are associated with structural and functional brain changes reflected by altered grey matter density, functional connectivity and brain activity during an associative memory task in areas related to memory (hippocampus) and depression (amygdala) and respective brain networks. We hypothesise that further brain areas are affected, especially those that showed altered glucose metabolism during hypothyroidism (posterior cingulate cortex, subcallosal cortex) and that brain changes are related to individual TPO-ab levels.

III Experimental Work

1 Publication 1: Quinque et al., 2013

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RESEARCH Open Access

Patient-reported outcomes in adequately treated hypothyroidism – insights from the German versions of ThyDQoL, ThySRQ and ThyTSQ

Eva M Quinque[1*], Arno Villringer[1,2], Juergen Kratzsch[3] and Stefan Karger[4]

Abstract

Background: Disease-specific patient-reported outcome measures (PROMs) have been developed as important research tools in the study of various diseases. For hypothyroidism there exist three validated disease-specific questionnaires in English: the Thyroid-Dependent Quality of Life Questionnaire (ThyDQoL), the Underactive Thyroid Symptom Rating Questionnaire (ThySRQ) and the Thyroid Treatment Satisfaction Questionnaire (ThyTSQ). We report psychometric properties of new German versions of the questionnaires including construct validity from two independent samples.

Methods: 230 envelopes with ThyDQoL, ThySRQ and ThyTSQ were given out to patients receiving levothyroxine for diagnosed hypothyroidism. Reliability and factor analyses were performed, correlations and hypothesised subgroup differences calculated to assess psychometric properties. Independently, 18 patients with treated hypothyroidism for autoimmune thyroiditis (Hashimoto's disease) and 18 healthy control subjects were enrolled in a clinical study. Participants filled in the above questionnaires alongside well-known generic PROMs, e.g. the Beck Depression Inventory, the 12-item Well-Being Questionnaire and the Short-Form-36. Two blood samples were taken. Groups were compared and correlations between disease-specific and generic instruments analysed. Relationships between PROMs and biochemically determined thyroid hormone status were investigated.

Results: 102 patients returned completed questionnaires (response rate 44%). The newly translated questionnaires had satisfactory psychometric properties. Cronbach's alpha was 0.92 for ThyDQoL, 0.81 for ThySRQ and 0.86 for ThyTSQ. For each of the questionnaires, a single factor structure explained the data best. Adequately treated patients with thyroid stimulating hormone levels in the upper normal range reported more symptoms in the ThySRQ. Those with autoimmune hypothyroidism reported being more bothered by depressive symptoms. Within the clinical sample, correlation with well-known generic instruments revealed good construct validity. In the clinical sample patients reported more symptoms in the ThySRQ, being more bothered by tiredness, higher depression and reduced well-being despite biochemically adequate treatment. Correlations between PROMs and biochemical thyroid hormone status revealed moderate though consistent associations.

Conclusions: Psychometric properties including construct validity of German versions of the ThyDQoL, ThySRQ and ThyTSQ are satisfactory. Feasibility and sensitivity in a clinical sample could be shown. We encourage the use of disease-specific PROMs in future studies as important additions to generic instruments in clinical research on hypothyroidism.

Keywords: Hypothyroidism, Hashimoto's thyroiditis, Patient-reported outcome measures (PROMs), Quality of life, Linguistic validation, Patient satisfaction

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Zusammenfassung

Hintergrund: Krankheitsspezifische Selbsteinschätzungsfragebögen, so genannte „patient-reported outcome measures"(PROMs), wurden als wichtige Forschungsinstrumente für verschiedene Erkrankungen entwickelt. Für die Hypothyreose wurden drei englischsprachige Fragebögen entwickelt: der Lebensqualitätfragebogen zur Schilddrüsenunterfunktion (ThyDQoL), der Symptomfragebogen zur Schilddrüsenunterfunktion (ThySRQ) sowie ein Fragebogen zur Behandlungszufriedenheit bei Schilddrüsenunterfunktion (ThyTSQ). Wir stellen psychometrische Eigenschaften der neuen deutschen Versionen dieser Fragebögen vor, inklusive Daten zur Konstruktvalidität aus zwei unabhängigen Stichproben.

Methoden: 230 Umschläge mit ThyDQoL, ThySRQ und ThyTSQ wurden an Patienten ausgegeben, die wegen einer gesicherten Hypothyreose mit Levothyroxin substituiert wurden. Mit Hilfe von Reliabilitäts- und Faktoranalysen, Korrelationsanalysen und hypothesengeleiteten Subgruppenvergleichen wurden die psychometrischen Eigenschaften der Fragebögen analysiert. 18 Patienten mit behandelter Hypothyreose autoimmuner Genese (Hashimoto Thyreoiditis) sowie 18 gesunde Kontrollprobanden nahmen an einer unabhängigen klinischen Studie teil. Die Teilnehmer füllten zu den oben genannten auch bekannte generische PROMs aus, darunter das Beck Depressions Inventar, den 12-Item Well-Being Questionnaire und den Short Form-36. Zwei Blutproben wurden entnommen. Die Gruppen wurden verglichen und Korrelationen zwischen krankheitsspezifischen und generischen Instrumenten sowie Zusammenhänge zwischen Fragebogendaten und Laborwerten berechnet.

Ergebnisse: 102 Patienten schickten ausgefüllte Fragebögen an die Autoren zurück (Rücklaufquote 44%). Die neu übersetzten Fragebögen zeigten gute psychometrische Eigenschaften. Cronbach's Alpha betrug 0.92 für den ThyDQoL, 0.81 für den ThySRQ und 0.86 für den ThyTSQ. Alle Fragebögen wurden am besten durch eine Einfaktorenlösung beschrieben. Patienten mit Thyroidea-stimulierendem Hormon (TSH) im oberen Normbereich berichteten mehr Symptome im ThySRQ und diejenigen mit autoimmuner Genese stärkere Beeinträchtigung durch depressive Symptome. Die Konstruktvalidität der Fragebögen konnte durch erwartungskonforme Korrelationen zu generischen Fragebögen in der klinischen Studie nachgewiesen werden. Die Patientengruppe berichtete trotz normwertigem TSH mehr Symptome im ThySRQ, mehr Beeinträchtigung durch Müdigkeit, sowie höhere Depressionswerte und geringeres Wohlbefinden als die gesunde Kontrollgruppe. Wir konnten moderate aber konsistente Zusammenhänge zwischen den Fragebogendaten und den Laborwerten finden.

Schlussfolgerung: Die psychometrischen Eigenschaften der Fragebögen ThyDQol, ThySRQ und ThyTSQ sowie ihre Konstruktvalidität sind zufriedenstellend. Machbarkeit und Sensitivität der Fragebögen in einer klinischen Studie konnten gezeigt werden. Die krankheitsspezifischen PROMs stellen eine wichtige Ergänzung zu den generischen Instrumenten dar, mit potentiellem Nutzen für die zukünftige klinische Forschung auf dem Gebiet der Hypothyreose.

Background

Hypothyroidism or subclinical hypothyroidism affects 4 to 21% of the female population and 3 to 16% of the male population [1]. Standard treatment for the highly prevalent condition is replacement of thyroid hormone by levothyroxine, artificial free thyroxine (fT4) [2]. The definition of the targeted normal range of thyroid hormone level is, however, still under debate [3-5]. Moreover, it has been reported that among patients receiving this treatment, well-being is reduced even if euthyroidism is reestablished [6,7].

It is still an open issue where patients' reports of unwanted symptoms result from [8]. Explanations discussed are independent effects of thyroid autoimmunity, the most common cause of hypothyroidism [9-11], insufficient normalisation of thyroid hormone levels at target tissues such as the brain despite normal serum hormone levels [12], selection bias in seeking health care [13] or reactive processes to the awareness of having a chronic disease [14]. Disentangling these possible causes has important implications for treatment targets in this large patient group. Crucial for successfully addressing the above issue is the use of appropriate instruments to measure patient-reported outcomes. It is important to differentiate between perceived health status, psychological well-being and quality of life as well as between generic and disease-specific instruments [15,16]. All are valid and important constructs to address patient-reported outcomes but should be carefully distinguished to avoid misleading interpretation of results. Symptom load has for example often been interpreted as quality of life although perceived symptoms may or may not influence quality of life in an individual [15,16]. Health status is often confusingly referred to as health-related quality of life [17]. It has been shown for several clinical conditions such as peripheral arterial disease or heart failure that disease-specific

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questionnaires are more sensitive to change [18,19]. However, most studies still use exclusively generic questionnaires and often self-constructed symptom lists to assess patient-reported outcomes in hypothyroidism, so reducing sensitivity to subtle effects and comparability across studies [20-23].

The first hypothyroidism-specific instruments have been developed and validated in recent years including the Thyroid-Dependent Quality of Life Questionnaire (ThyDQoL) [24,25]. The ThyDQoL measures the impact of hypothyroidism on quality of life in general and in selected domains tailored to the disease and to individual realities by including importance ratings for each domain. The Underactive Thyroid Symptom Rating Questionnaire (ThySRQ) is in contrast a measure of hypothyroidism-related symptoms and symptom bother [25]. Finally, the Thyroid Treatment Satisfaction Questionnaire (ThyTSQ) measures disease-specific treatment satisfaction [24,26]. It is designed to cover hypothyroidism-specific aspects such as satisfaction with current medication and dose.

All three hypothyroidism-specific questionnaires have been developed and validated in English and use of the questionnaires in any other language needs validation in an independent sample to examine psychometric validity. Although necessary, this is a demanding and time consuming procedure, possibly contributing to the paucity of validated translations. However, despite known advantages over the use of exclusively generic or non-validated instruments, the original questionnaires are also relatively new, which may account for the fact that they are not yet in widespread use. None of the validated hypothyroidism-specific questionnaires available has been evaluated for German so far, although interest in the field is high in German speaking countries [10,21-23,27].

We are thus introducing the first three hypothyroidism-specific PROMs in German to improve the array of tools available for future research. We provide detailed psychometric data including internal consistency and factor structure of the questionnaires, as well as hypothesised subgroup analyses. According to the literature we expect more negative reports in patients with thyroid stimulating hormone (TSH) in the upper normal range [28] and more negative reports in patients with hypothyroidism of autoimmune origin [10,27].

In addition, we have used the questionnaires in a clinical study including 18 adequately treated patients with hypothyroidism due to autoimmune thyroiditis (Hashimoto's disease) and 18 healthy control subjects. The study also included a number of well-known generic PROMs. Thereby, we were able to investigate construct validity of the new questionnaires and feasibility in a clinical context. We expect disease-specific and generic instruments to be moderately correlated because similar, though distinct, constructs are targeted. The clinical study included assessment of TSH, fT4, free triiodothyronine (fT3) as well as thyroid peroxidase antibodies (TPOAb) and thyroglobulin antibodies (TgAb). This design allowed investigation of the relationship between PROMs and biochemical thyroid hormone status.

Methods

Linguistic validation

Linguistic validation was performed for ThyDQoL, ThySRQ and ThyTSQ as previously described for similar instruments [29]. English originals were translated into German by two native German speakers, including a clinical endocrinologist (SK) and reconciled into a preliminary forward translation by a psychologist (EQ). The resulting forward translation was subsequently translated back into English by each of two native English speakers. Any discrepancies between original and back-translation were discussed with the developer's team and improvements made where necessary. The resulting draft translation was then used for cognitive debriefing interviews with five patients with hypothyroidism of different origins, recruited from the volunteer database of the Max Planck Institute for Human Cognitive and Brain Sciences (MPI) and reimbursed for their time. Example items of the final German versions can be found in Figure 1.

Scoring of questionnaires

The scoring for the newly translated questionnaires ThyDQoL, ThySRQ and ThyTSQ will be briefly summarised as explained in detail elsewhere [25,26]. The ThyDQoL starts with two overview items on present quality of life (present QoL) and impact of hypothyroidism on quality of life in general (impact on QoL). The first ranges from excellent (3) to extremely bad (−3), the second from very much better (-3) to worse (1) without hypothyroidism. The 18-item main questionnaire asks for impact of hypothyroidism on various domains of life such as work life or sex life (see [25] for a complete list of domains). Nine of the domains have a "not applicable" option to guarantee individual relevance of the items. For each domain, if applicable, respondents rate whether life in this domain would be very much better (−3) to worse (1) without hypothyroidism. In addition, respondents rate the importance of the respective domain from very important (3) to not at all important (0). A weighted domain impact score is calculated by multiplying both ratings for each domain resulting in scores ranging from −9 (maximal negative impact of hypothyroidism on quality of life) to 3 (maximal positive impact of hypothyroidism). These scores can be summed into an Average Weighted Impact Score (AWI–18, ranging from −9 to 3) by summing all domain weighted impact scores and dividing the result by the number of applicable and completed domains if at least half of

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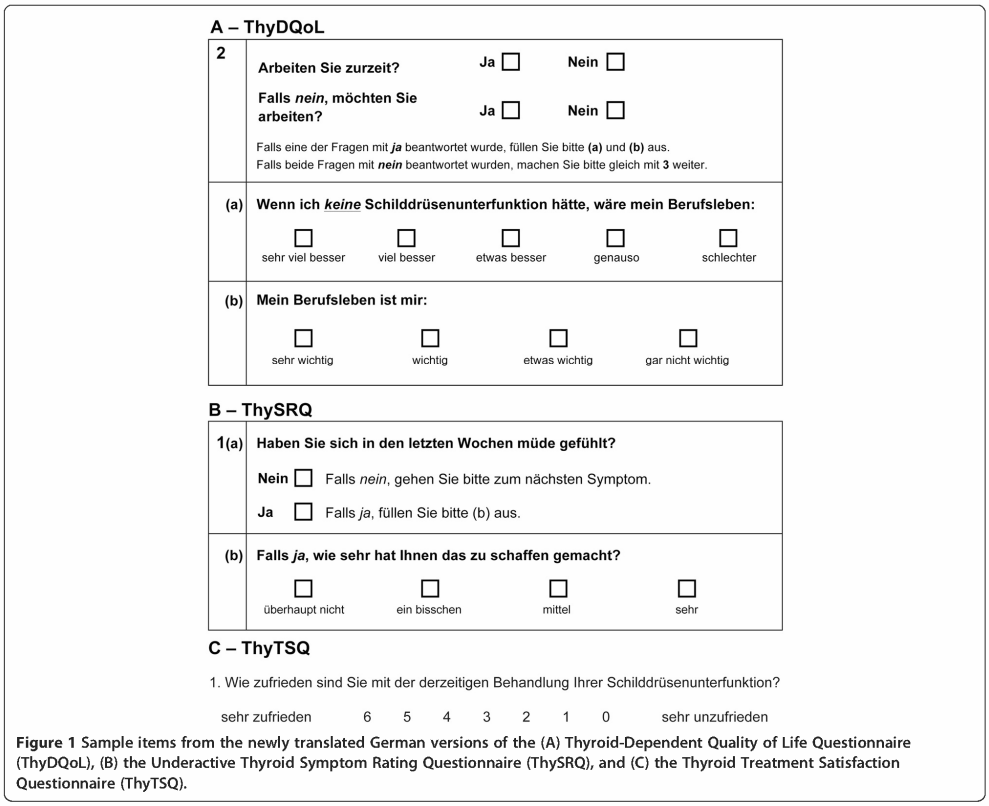

Figure 1 Sample items from the newly translated German versions of the (A) Thyroid-Dependent Quality of Life Questionnaire (ThyDQoL), (B) the Underactive Thyroid Symptom Rating Questionnaire (ThySRQ), and (C) the Thyroid Treatment Satisfaction Questionnaire (ThyTSQ).

the items are applicable and completed. In addition, the AWI–14 can be calculated by excluding four items which overlap with the ThySRQ. Finally, a free comments section at the end allows respondents to indicate further domains not covered in the questionnaire.

The 15-item ThySRQ requires a yes/no response on whether each of the given hypothyroidism-related symptoms such as feeling cold or weight gain has been experienced in recent weeks (see [25] for a complete list of symptoms). If experienced, the amount of bother from the symptom is rated from not at all bothered (0) to very much bothered (3). If a symptom is not experienced bother rating for this item is zero.

The 7-item ThyTSQ covers several aspects of current treatment satisfaction, e.g. general satisfaction with or convenience of treatment (see [24] for a complete list of items). Items range from very satisfied (6) to very dissatisfied (0) or equivalent. Range of the summed score is 0–42. The ThyTSQ also offers a free comment section at the end.

Patients and procedures

For the validation study 230 envelopes with questionnaires were given out by local endocrinologists and the outpatient department of the Clinic for Endocrinology at University Hospital Leipzig. The questionnaires were accompanied by a motivating letter and a questionnaire on basic sociodemographic and disease details as shown in Table 1.

In the clinical study 25 patients with treated hypothyroidism due to autoimmune thyroiditis independent of the validation sample as well as 27 healthy control subjects were enrolled after written informed consent. Patients were recruited via internet advertisement, local endocrinologists and the MPI's volunteer database. Healthy control subjects were recruited via the database alone. The database consists of volunteers recruited via the MPI's website or via advertisement for former non-clinical studies. All participants were reimbursed for their time. The research protocol of both studies was approved by the ethics committee of the University of Leipzig and

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Table 1 Characteristics of the sample for psychometric validation

Sample characteristics	Mean	SD	Range	N
Age (years)	43.5	16.3	19-75	101
Sex (female/male)		93/8		101
Marital Status		Single/Married/Divorced/Widowed		101
		37/48/12/4		
TSH (mU/l)	2.20	3.95	0.02-35.0	90
Treatment duration (years)	7.48	6.94	0.2 - 39.0	96
Current dose LT4* (μg/day)	97.9	42.4	0-250	99
Cause of hypothyroidism	Autoimmune thyroiditis			75
	Thyroidectomy for cold nodules or nodular goiter			13
	Ablative treatment for hyperthyroidism			7
	Lithium treatment			1
	Thyroidectomy – cause not specified			2
	Missing			3

*two participants additionally received 7.5/10 μg T3 respectively **comorbid conditions with potential influence occurring in more than one participant: depression (4), migraine (4), diabetes (4), asthma (3), heart disease (2), polycystic ovary syndrome (2) and pregnancy (2); several patients had multiple comorbid conditions. None of the non-reported rare comorbidities was a psychiatric illness. Abbreviaton: SD – Standard Deviation.

was in accordance with the latest version of the Declaration of Helsinki.

Patients in the clinical study filled in the three newly translated questionnaires ThySRQ, ThyDQoL and ThyTSQ alongside well-known and validated generic instruments of mood and well-being as part of a larger study. Healthy control subjects completed the same questionnaires except for the ThyTSQ and most parts of the ThyDQoL because they are not meaningful to healthy subjects. The ThyTSQ explicitly asks questions about the satisfaction with the treatment that healthy subjects do not receive and the ThyDQoL asks for the quality of life in several domains in the style "If I did not have underactive thyroid, my working life would be…". However, the first question of the ThyDQoL asks for general quality of life and is thus meaningfully answerable for healthy control subjects. The ThySRQ asks for symptoms independent of the disease, e.g. "Have you felt tired in recent weeks?" which is also meaningful to healthy control subjects. Please note however that the use of any of the Thy questionnaires in a healthy population has not been validated yet and our study can thus only provide preliminary results. General perceived physical and mental health status was measured by the Short Form-36 (SF-36, [30]). Higher values in the range of 0–100 in the two subscales physical and mental health stand for better perceived health. General mental strain was assessed by the Symptom Check List (SCL-90-R, [31]), higher values in the range of 0–4 meaning higher strain. General well-being was measured with the 12-item Well-Being Questionnaire (WBQ-12, [32]), higher values in the range of 0–36 indicate better well-being. Generic instruments were given first to prevent their interpretation being influenced by the content of the disease-specific instruments. Depression was assessed by a questionnaire, the Beck Depression Inventory (BDI, [33]) and a structured interview, the Hamilton Depression Scale (HDS, [34]), in both instruments higher values mean greater amounts of depression. Cut-off scores for clinical depression are eleven points for the BDI and seven points for the HDS.

Basic sociodemographic data were asked for in written form. In addition, fasting blood samples were taken in the morning to assess TSH, fT3, fT4, TPOAb and TgAb. Blood samples were analysed at the Institute for Laboratory Medicine of the University Hospital Leipzig by the fully automated Roche cobas system (Roche, Basel, Switzerland).

Statistical analysis

All questionnaire items entering psychometric evaluation were checked for normal distribution by investigating histograms and skewness scores. Normality as checked by histograms and Kolmogorov-Smirnov tests for normal distribution was not given for most variables. The skewness threshold of ±2.58 [26] was slightly exceeded by two items (ThyDQoL item getting out −3.2, ThySRQ item appetite 3.9). Reflect and log transformation did not significantly reduce skewness in these variables (getting out 2.7, appetite 3.7), so that statistics were calculated on the original data, but nonparametric tests were chosen for statistical analyses. Spearman's rho and Mann–Whitney U-tests (MW-U-tests) were chosen for correlations and group comparisons respectively and parametric tests were employed for exploratory analyses only when no comparable nonparametric test was available, such as partial correlation or analysis of covariance (ANCOVA). Chi-square tests were used for comparison of nominal

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data. Statistical significance was accepted if $p < 0.05$ for all analyses and Bonferroni correction for multiple testing was applied where necessary.

Results

Sample evaluation study

102 questionnaires were returned anonymously in stamped addressed envelopes to the first author between September 2011 and November 2012; the response rate was 44%. Due to the anonymous design of the study no data were available on the non-responders. One participant reported not being treated yet and was thus excluded from any analysis. Among those reporting TSH values, 68 were within the normal range of 0.4–4.0 mU/l ([35], see Table 1). Seventy-one participants reported no or only symptom-free comorbid conditions such as hypertension. Thirty reported comorbidities likely to influence results (see Table 1) and subgroup comparisons were controlled for these influencing comorbidities.

Psychometric evaluation of the newly translated instruments

As numbers for not applicable options were high in the ThyDQoL (0% for family to 45% for depression) they were coded as zero for factor and reliability analyses but as not applicable for calculation of the AWI-18 and AWI-14. One participant did not fill in the ThyDQoL, for all others the AWI-18 and AWI-14 could be calculated. Completion rate in the remaining sample was between 95 and 100% per item. Genuinely missing items were excluded for factor and reliability analysis, leaving 79 complete datasets for analysis. Unforced factor analysis on the 18 weighted-impact scores produced 4 factors, but the Varimax rotated factor loadings did not allow a meaningful interpretation. The screeplot suggested a single most important factor explaining 45% of the variance. A forced one-factor solution revealed that all variables loaded saliently (above 0.30) on the single factor, ranging from 0.36 (weight) to 0.82 (friendship and physical ability) and all but the weight item even above 0.40, implying robust findings [25]. Cronbach's alpha was 0.92, and all variables had an acceptable corrected item-total correlation above 0.20, ranging from 0.35 (weight) to 0.78 (physical ability). Thirty respondents used the free comments section. Eleven mentioned comorbidities not included in the present questionnaire. Six of forty-nine, or 12% of women of childbearing age (18–40 years) mentioned involuntary infertility. Fertility may thus be a potentially relevant domain for inclusion in future versions of the ThyDQoL at least when targeting younger women.

98 complete datasets were available for factor and reliability analysis of the ThySRQ. Two participants missed one item each and one participant missed the final page (four items). Unforced factor analysis on the symptom bother ratings revealed five factors. However, the factors could not be interpreted in a meaningful way and the screeplot pointed towards a single factor solution. The forced 1-factor solution explained 28% of the variance and variables loaded between 0.20 (constipation) and 0.74 (concentration). Only constipation loaded below 0.30 and nails (0.36) below 0.40 on the single factor. Cronbach's alpha was 0.81. All variables except for constipation (0.13) had a corrected item-total correlation above 0.20 ranging from 0.28 (nails) to 0.63 (concentration).

99 complete datasets were available for factor and reliability analysis of the ThyTSQ. One participant missed a single item and one the whole questionnaire. Unforced factor analysis produced a single factor as also suggested by the screeplot. This factor explained 57% of the variance. All variables loaded robustly (above 0.40) on this factor, ranging from 0.48 (convenience) to 0.89 (how well working). Cronbach's alpha was 0.86. All variables had a corrected item-total correlation above 0.20, ranging from 0.37 (convenience) to 0.80 (how well working). Twenty-one participants used the free comments section, but did so only to stress points already covered by the ThyTSQ.

Descriptive results and comparison to the English originals

The current sample (n = 101) was significantly younger than the original sample (mean = 44 vs. 55 years, one-sample t-test $p < 0.001$; all comparison data from [25]) but comparable in the distribution of comorbidities. The present sample mean of the AWI-18 (n = 100) was −1.50 (SD = 1.4, range −5.7 to 0), indicating negative impact of hypothyroidism on quality of life. People reported significantly less impact on quality of life than in the original sample (mean = −3.11; $p < 0.001$). General quality of life was rated as "good" (n = 100, mean = 0.91, SD = 0.95, range −2 to +3) not significantly different from the original sample mean = 0.89 ($p > 0.1$) and general impact of hypothyroidism on quality of life was rated as "a little better without hypothyroidism" (n = 98, mean = −0.88, SD = 0.76, range −3 to 0 vs. mean = −1.25; $p < 0.001$), less negative than in the original sample. Mean number of reported symptoms was 5.6 (n = 98, SD = 3.3, range 0–13), which is significantly less than in the original sample (mean = 7.4; $p < 0.001$). Sample mean of the TSQ sum score was 31.6 (n = 99, SD = 7.6, range 10–42), comparable to the reference finding [25] (mean = 32.5; $p > 0.1$).

Intercorrelations

Correlations were performed between the three new questionnaires to investigate construct validity. For better comparability between correlations, data were excluded listwise, resulting in n = 91 complete datasets entered into all analyses reported in Table 2.

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Table 2 Spearman correlations between the ThyDQoL, ThyTSQ, and ThySRQ items

ThyDQoL indices	ThyDQoL AWI-18	ThyDQoL AWI-14	ThyDQoL Present QoL	ThyDQoL Impact on QoL	ThyTSQ sumscore
AWI-18	———	0.97*	0.51*	0.75*	0.57*
AWI-14	———	———	0.52*	0.72*	0.56*
Present QoL	———	———	———	0.30*	0.48*
Impact on QoL	———	———	———	———	0.55*
ThySRQ symptom bother ratings					
Tiredness	−0.39**	−0.39**	−0.28**	−0.34**	−0.37**
Weight gain	−0.38**	−0.33**	−0.14 n.s.	−0.34**	−0.27*
Cold	−0.23*	−0.20 n.s.	−0.27*	−0.14 n.s.	−0.07 n.s.
Constipation	−0.13 n.s.	−0.09 n.s.	−0.08 n.s.	−0.18 n.s.	−0.16 n.s.
Hair	−0.29*	−0.32**	−0.27*	−0.24*	−0.26*
Skin	−0.35**	−0.35**	−0.26*	−0.31**	−0.26*
Nails	−0.31**	−0.31**	−0.23*	−0.34**	−0.21 n.s.
Appetite	−0.25*	−0.23*	−0.19 n.s.	−0.11 n.s.	−0.02 n.s.
Hearing	−0.14 n.s.	−0.12 n.s.	−0.08 n.s.	−0.13 n.s.	0.03 n.s.
Voice	−0.32**	−0.30*	−0.30**	−0.05 n.s.	−0.21*
Speech	−0.37**	−0.35**	−0.22*	−0.25*	−0.13 n.s.
Memory	−0.22*	−0.20 n.s.	−0.22*	−0.20 n.s.	−0.06 n.s.
Concentration	−0.42**	−0.43**	−0.47**	−0.26*	−0.43**
Giddy	−0.33**	−0.32**	−0.23*	−0.31**	−0.22*
Depressed	−0.48**	−0.46**	−0.56**	−0.27*	−0.45**

*$p < 0.05$, **$p < 0.0033$ corrected for multiple testing within ThySRQ symptom bother ratings. Abbreviations: ThyDQoL – Thyroid-Dependent Quality of Life Questionnaire, ThySRQ – Underactive Thyroid Symptom Rating Questionnaire, ThyTSQ – Thyroid Treatment Satisfaction Questionnaire, AWI – Average Weighted Impact Score, QoL – Quality of Life.

AWI-18 and AWI-14 showed a similar correlation pattern to the ThySRQ items, so that spurious intercorrelations by overlapping items between AWI-18 and ThySRQ can be ruled out and the original AWI-18 will be used for all further analyses. The single-item impact on QoL is highly correlated with the multiple-item AWI-18, so that we will use only the AWI-18 for further analyses. Correlations between both AWI scores and ThySRQ symptom bother ratings ranged between −0.09 and −0.48 all in the expected direction but of small to moderate size, implying that symptom bother and impact on quality of life are related but distinct constructs. Treatment satisfaction as measured by the ThyTSQ showed low to moderate mainly negative correlations with the ThySRQ symptom bother ratings (range 0.03 to −0.45) and moderate positive correlations (range 0.48 to 0.57) with the different ThyDQoL indices. Treatment satisfaction is thus meaningfully related to but separable from the other two disease-specific PROMs.

Forward models of multiple regression analysis revealed that among those ThySRQ items correlating significantly with present QoL, depression ($F(1,97) = 42.5$, $p < 0.001$) and feeling cold ($F(2,96) = 25.1$, $p < 0.001$) significantly predicted present QoL, explaining 34% of the variance. The impact of hypothyroidism on quality of life (AWI-18) was significantly predicted by depression ($F(1,97) = 33.9$), speech problems ($F(2,96) = 33.4$), nail problems ($F(3,95) = 27.0$) and tiredness ($F(4,94) = 22.3$), explaining 49% of the variance, all $p < 0.001$. Analogously, depression ($F(1,96) = 24.2$) and tiredness ($F(2,95) = 15.7$) significantly predicted treatment satisfaction, explaining 25% of the variance, all $p < 0.001$. The ThySRQ depression item alone explained 31% of the variance of present QoL, 26% of variance of the impact of hypothyroidism on QoL and 20% of the variance of treatment satisfaction.

Subgroup analyses

Subgroup analyses were performed with pairwise deletion of missing items in order to reduce information loss for the smaller sample sizes considered here. Within the group of adequately treated patients (n = 68) symptom bother ratings of depression ($r = 0.47$, $p < 0.001$) and hair ($r = 0.37$, $p < 0.002$) correlated significantly with TSH, depression even after Bonferroni correction ($p < .0033$). Additionally, age correlated negatively with TSH ($r = -0.39$, $p < .002$). Therefore, an exploratory partial correlation controlling for age was performed yielding the exact same pattern as above, depression ($r = 0.44$, $p < 0.001$) and hair ($r = 0.42$, $p < 0.001$) bother ratings correlating significantly

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even after Bonferroni correction. Similar results were obtained when considering either the full sample with available TSH values (n = 91) or only adequately treated patients with autoimmune thyroiditis (n = 51). Again depression was the only correlation surviving Bonferroni correction ($r = 0.32$, $p < 0.002$) in the full sample or just missing Bonferroni correction in the group with autoimmune thyroiditis ($r = 0.37$, $p < 0.009$).

Comparing adequately treated patients split by median TSH (n = 34 in both groups, TSH median = 1.44 mU/l) revealed that patients in the lower range were older (49 vs. 40 years; $p < 0.05$) but groups did not differ in the amount of comorbidities ($p > 0.1$), explicitly depression (n = 2 in each group) nor gender ($p > 0.1$) or type of diagnosis (autoimmune vs. other $p = 0.1$). Those in the upper TSH range reported significantly more bother by hair (1.3 vs. 0.5; $p < 0.006$), depression (1.4 vs. 0.6; $p < 0.003$) and concentration (1.2 vs. 0.7; $p < 0.05$) symptoms and overall more symptoms in the ThySRQ (6.4 vs. 4.8; $p < 0.05$). Exploratory ANCOVAs controlling for age revealed that only hair ($p < 0.007$) and depression symptom bother ($p < 0.005$) differed significantly between TSH groups independently of age. After Bonferroni correction, only depression symptom bother was significant before controlling for age.

Among the adequately treated participants with known cause of hypothyroidism those with autoimmune thyroiditis (n = 51) were compared to those reporting a different diagnosis (n = 16). Groups did not differ in gender and comorbidities, explicitly not in depression (n = 2 in each group), but in TSH (autoimmune 1.63 mU/l vs. other 1.05 mU/l; $p < 0.01$) and age (40.9 vs. 57.3 years; $p < 0.001$). The group with autoimmune thyroiditis reported significantly higher bother ratings for depression (1.22 vs. 0.33; $p < 0.009$) as well as reduced treatment satisfaction (30.7 vs. 35.4; $p < 0.05$) before Bonferroni correction (corrected $p < .0033$). In an exploratory ANCOVA with age and TSH as covariates the group effect on depression remained significant on an uncorrected level.

Clinical sample

Two of the original 25 treated patients did not complete the study for reasons unrelated to disease status. Five of the remaining 23 treated patients had to be discarded because of unstable doses or persistently heightened TSH levels. Autoimmunity was either proven by positive TPOAb and/or TgAb or hypoechogenicity at thyroid ultrasound from clinical records. Nine of the originally 27 healthy control subjects had to be discarded for either positive TPOAb, TgAb or heightened TSH levels in two consecutive blood samples taken about 3 months apart, leaving 18 participants in both groups, see Table 3.

Correlations between the newly translated hypothyroidism-specific questionnaires and previously validated and published questionnaires on mood and well-being revealed that all correlated in the expected direction although only a subset reached significance, see Table 4. Correlations are between small and moderate.

Comparison of clinical groups

ThyDQoL and ThyTSQ are only meaningful to treated patients as explained above. In a clinical study including a control group only the ThySRQ and the ThyDQoL present QoL item are thus meaningful for group comparisons, although their use in a healthy sample still needs independent evaluation and reported results here are of preliminary nature. Comparison of the two groups in the clinical study revealed several significant results or statistical trends, see Table 5. Among the ThySRQ symptom bother ratings only tiredness ($p < 0.003$) reached significance after Bonferroni correction. Results for the other ThySRQ symptoms are not shown.

Correlation with thyroid parameters

Higher fT4 in the group of treated patients was correlated significantly with lower impact of hypothyroidism on quality of life as measured by AWI-18 ($r = 0.51$) before Bonferroni correction. None of the other thyroid parameters correlated significantly with the questionnaire data. However, in an exploratory inspection of the full correlation table, the majority of correlations were in the expected direction of more negative reports with worse thyroid status for TSH, fT4 and TPOAb and between the generic instruments and TgAb, see Table 6. Interestingly, for the disease-specific questionnaires, this pattern was completely reversed for fT3 and TgAb.

Discussion

Validity of the newly translated questionnaires

All three questionnaires reached psychometric validity comparable to the English originals and most importantly, mostly reached the standards as laid out in [25] (factor loadings on single factor >0.4, Cronbach's Alpha >0.8, Corrected item-total correlations >0.2). The ThySRQ only slightly missed the robustly salient factor loading for the items constipation and nail problems as similarly reported for the original ThySRQ [25] and the weight gain item from the ThyDQoL missed the robust 0.4 loading, but still loaded saliently. The current sample was on average 10 years younger than the original sample and reported slightly better status on ThyDQoL and ThySRQ, but similar treatment satisfaction in the ThyTSQ. However, the current sample still reported a negative impact of hypothyroidism and an average of six symptoms despite biochemically adequate treatment, which is in line with previous literature reporting residual complaints [6,7]. Treatment satisfaction was in the moderately positive range but with room for improvement.

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Table 3 Characteristics of the clinical sample with treated hypothyroidism in proven autoimmune thyroid disease and the healthy control group

Sample characteristics	Treated patient group n = 18			Healthy control group n = 18			*p*-value
	Mean	SD	Range	Mean	SD	Range	
Age (years)	32.2	9.60	[18–54]	32.1	8.90	[18–53]	>0.1
Sex (female/male)		16/2			16/2		>0.1
TSH (mU/l)*	1.96	1.42	[0.2 - 5.3]	2.63	0.95	[0.9 - 5.0]	<0.05
fT3 (pmol/l)*	4.28	0.62	[3.2 - 5.6]	4.72	0.83	[3.2 - 6.3]	=0.07
fT4 (pmol/l)*	18.2	1.61	[14–21]	15.6	2.24	[14–23]	<0.001
TPOAb (U/ml)*	140	132	[9–457]	10.2	2.14	[7–14]	<0.001
TgAb (U/ml)*	313	604	[5–2424]	17.3	7.47	[5–36]	<0.001
Treatment duration (years)	4.41	4.21	[1–15]	not applicable			
Current dose (μg/day)	96.5	29.6	[50–150]				

*laboratory values of initial blood sample, reference range was 0.4-4.0 for Thyroid Stimulating Hormone (TSH), 3.1-6.79 for free Triiodothyronine (fT3), 12.8-20.4 for free Thyroxine (fT4), < 34 for Thyroid Peroxidase Antibodies (TPOAb) and < 33 for Thyroglobulin Antibodies (TgAb) [4,35]. Patients with slightly altered laboratory values that spontaneously recovered to the normal range in a second blood sample taken 3–6 months later were considered as thyroid healthy or adequately treated and included into the analysis. Abbreviaton: SD – Standard Deviation.

Due to the anonymous design of the study, which allowed maximal data privacy and reduced bias due to social desirability effects, no data were available on the non-responders. However, our sample is comparable to similar questionnaire studies on PROMs in treated hypothyroidism, which include data on non-responders according to age, sex ratio, cause of hypothyroidism, percentage normal TSH levels, and disease duration. Moreover, comparison of responders to non-responders in these similar studies revealed only a slight bias with respect to age and sex ratio [6,25,26]. Furthermore, our sample showed a broad range and variance of disease parameters (see Table 1) and reported severity of impairment so that it is unlikely that the responders form a highly selective group within the population of treated patients with hypothyroidism.

Intercorrelation of ThySRQ items with ThyDQoL replicated previous findings [25] again showing the AWI-18 can be used together with the ThySRQ without causing spurious correlations by four shared items. We here additionally included the ThyTSQ and found comparable effect sizes of correlations to ThySRQ items and AWI-18 as already shown between the other two questionnaires, implying that the three questionnaires measure related but sufficiently separate constructs. Among the ThySRQ items, it was depression that explained most variance to predict present quality of life, impact of hypothyroidism on quality of life and treatment satisfaction, so depressive symptoms should be given special attention when evaluating patient reports.

Higher depression bother rating was robustly associated with higher TSH across adequately treated patients or the whole sample. Interestingly, although no further results reached significance, the large majority of correlations above 0.20 were in the direction of lower normal TSH values with better patient-reported outcomes. Our results are in line with the literature proposing to treat patients towards TSH levels in the lower normal range [5,28]. However, treatment outcomes other than patient reports have to be considered, such as effects on heart function or cognition [8,36], especially in patients beyond the age of 75 [37], in order to achieve optimal treatment for the individual patient. Patients reporting autoimmune thyroid disease as opposed to other causes for hypothyroidism

Table 4 Spearman correlations between hypothyroidism-specific and generic patient-reported outcome measures in treated patients

Specific vs. generic instruments	ThyDQoL present QoL	ThyDQoL AWI-18	ThySRQ symptom number	ThyTSQ sumscore
Short Form-36 physical	0.40	0.33	−0.15	0.33
Short Form-36 mental	0.29	0.30	−0.39	0.31
WBQ-12	0.53*	0.64*	−0.61*	0.47
SCL-90-R	−0.48*	−0.53*	0.58*	−0.51*
Hamilton Depression Scale	−0.53*	−0.49*	0.45*	−0.74**
Beck Depression Inventory	−0.56*	−0.62*	0.29	−0.61*

*$p < 0.05$, **$p < 0.002$ Bonferroni corrected. Abbreviations: WBQ – Well-Being Questionnaire, SCL – Symptom Check List, ThyDQoL – Thyroid-Dependent Quality of Life Questionnaire, ThySRQ – Underactive Thyroid Symptom Rating Questionnaire, ThyTSQ – Thyroid Treatment Satisfaction Questionnaire, AWI – Average Weighted Impact Score, QoL – Quality of Life.

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Table 5 Patient-reported outcome measures in patients with autoimmune thyroid disease and matched healthy control subjects

Patient-reported outcomes	Treated patient group n = 18		Healthy control group n = 18		*p*-value
	Mean	SD	Mean	SD	
ThySRQ symptom number*	5.44	2.83	2.93	2.63	<0.05
ThyDQoL Present QoL	1.17	0.92	1.61	0.78	n.s.
Hamilton Depression Scale	6.00	3.76	3.72	2.35	=0.06
Beck Depression Inventory*	5.67	4.65	3.00	3.55	<0.05
Short Form-36 physical	54.80	8.50	56.07	5.07	n.s.
Short Form-36 mental	42.93	11.14	49.28	6.81	=0.09
WBQ-12	23.72	5.36	27.78	4.44	<0.05
SCL-90-R	0.39	0.28	0.35	0.33	n.s.
ThySRQ tiredness*	1.67	0.91	0.60	0.63	<0.0033

*Only available in n = 15 healthy control subjects, excluding the same subjects for all analyses does not change any of the results. Abbreviations: SD – Standard Deviation, WBQ – Well-Being Questionnaire, SCL – Symptom Check List, ThyDQoL – Thyroid-Dependent Quality of Life Questionnaire, ThySRQ – Underactive Thyroid Symptom Rating Questionnaire, QoL – Quality of Life.

reported significantly more bother by depressive symptoms, in line with claims of an independent role for autoimmunity in residual symptoms [9-11].

Clinical study

Correlations between hypothyroidism-related questionnaires and generic instruments in the clinical sample were all in the expected direction. The effect size was small to moderate, meaning that the new disease-specific questionnaires do share common variance with the generic instruments but also hold distinct variance to the more generic instruments. They are thus valid additions for future studies. Those disease-specific questionnaires applicable also to healthy control subjects (ThySRQ and the present QoL item of ThyDQoL) proved to be as sensitive in detecting subtle differences in small clinical samples as the well-established generic instruments. It is remarkable that descriptively all results were in the expected direction of more negative patient reports in the treated patient group adding to the literature on residual symptoms despite biochemically adequate treatment [6,7].

Table 6 Spearman correlations between laboratory values and patient-reported outcomes in the group of treated patients

Laboratory results - patient-reported outcomes	TSH	fT3	fT4	TPOAb	TgAb
ThyDQoL AWI-18	−0.23[1]	−0.04[2]	0.51[1*]	−0.19[1]	0.07[2]
ThyDQoL Present QoLresent QoL	−0.23[1]	−0.28[2]	0.30[1]	−0.01[1]	0.19[2]
ThySRQ number symptoms	0.26[1]	0.09[2]	−0.31[1]	0.15[1]	−0.21[2]
ThyTSQ	−0.17[1]	−0.39[2]	0.39[1]	−0.02[1]	0.05[2]
Short Form-36 physical	−0.37[1]	−0.07[2]	0.21[1]	−0.33[1]	−0.04[1]
Short Form-36 mental	0.16[2]	−0.21[2]	−0.08[2]	0.27[2]	0.20[2]
SCL-90-R	0.05[1]	0.02[2]	−0.23[1]	0.24[1]	0.24[1]
WBQ-12	−0.15[1]	0.05[1]	0.36[1]	−0.12[1]	−0.03[1]
Hamilton Depression Scale Scale	0.19[1]	0.10[2]	−0.40[1]	0.43[1]	0.27[1]
Beck Depression Inventory	−0.12[2]	−0.16[1]	−0.40[1]	0.06[1]	0.16[1]

*$p < 0.05$, [1]in the expected direction, [2]against the expected direction. Abbreviations: WBQ – Well-Being Questionnaire, SCL – Symptom Check List, ThyDQoL – Thyroid-Dependent Quality of Life Questionnaire, ThySRQ – Underactive Thyroid Symptom Rating Questionnaire, ThyTSQ – Thyroid Treatment Satisfaction Questionnaire, AWI – Average Weighted Impact Score, QoL – Quality of Life, TSH – Thyroid Stimulating Hormone, fT3 – free Triiodothyronine, fT4 – free Thyroxine, TPOAb – Thyroid Peroxidase Antibodies, TgAb – Thyroglobulin Antibodies.

Only a few correlations between hormone levels and patient-reported outcomes in treated patients reached significance, possibly due to the low power of the study. However, TSH, fT4 and TPOAb consistently correlated in the expected direction of more negative PROMs with worse biochemical status, and being most pronounced in the disease-specific questionnaires. This, though necessarily preliminary, favours a subtle relationship between certain biochemical thyroid hormone markers within the normal range and PROMs in treated patients. In contrast, fT3 and TgAb showed a reverse (fT3) or inconsistent (TgAb) relationship to PROMs, in line with clinical routine that does not consider them as valid markers of thyroid hormonal status. Interestingly, TPOAb were equally related to patient-reported outcomes as TSH and fT4. This is in line with literature considering autoimmunity as an independent factor for treatment outcome [10,27].

We acknowledge the small sample size of the clinical study. However, our sample size is comparable to other clinical studies [23] in the field and it is thus important to show that instruments are also sensitive enough to detect group differences in small samples as shown here. Among the Thy questionnaires, we found significant group differences for the number of reported symptoms in the ThySRQ and for the tiredness item, but not for the overall quality of life question in the ThyDQoL. These results should be interpreted with caution as the use of the questionnaires in healthy control subjects has not been validated so far, but are still of preliminary interest to encourage further validation and use of the questionnaires in cross-sectional studies including healthy control subjects. Our clinical sample was of a young age range in order to exclude effects of aging and comorbidities on the data, and thus does not contribute to research on possible specificities of hypothyroidism in the elderly such as beneficial effects on the coronary system in patients over 85 years, which leads to reduced mortality [37]. Therefore, treatment targets a slightly higher TSH value in the very elderly. Studies on quality of life and perceived symptoms in this age group are not known to us and would be

difficult to interpret, because symptoms of hypothyroidism greatly resemble those of general aging and therefore are prone to confusion in elderly subjects [37]. Our findings therefore cannot be extended to the very elderly. Most importantly however, the 10-year age difference and stronger severity of reported symptoms between our evaluation sample and the original one [25] did not influence the findings on the psychometric properties of the questionnaires, so that our main finding, the sufficient psychometric quality of the questionnaires can be generalised to the use in young and middle aged cohorts.

Finally, the majority of participants were female, owing to the gender bias in the prevalence of the disease, as a consequence of which our results can only be generalised reliably to the female population and the study included only patients already on treatment for hypothyroidism, so that the interpretation is confined to the group of already treated patients.

Conclusions

The three hypothyroidism-specific PROMs ThyDQoL, ThySRQ and ThyTSQ, introduced here as new German translations, show good psychometric properties and meaningful relations to TSH. In a small clinical sample we have found preliminary evidence for construct validity with well-established generic instruments and for a relationship to thyroid laboratory measures. We thus recommend them for use in clinical studies and encourage further linguistic validation into other languages to improve available research tools as a prerequisite for progress in clinical research on hypothyroidism.

Abbreviations

ThyDQoL: Thyroid-Dependent Quality of Life Questionnaire; ThySRQ: Underactive Thyroid Symptom Rating Questionnaire; ThyTSQ: Thyroid Treatment Satisfaction Questionnaire; PROMs: Patient-reported outcome measures; TSH: Thyroid Stimulating Hormone; fT3: free Triiodothyronine; fT4: free Thyroxine; TPOAb: Thyroid Peroxidase Antibodies; TgAb: Thyroglobulin Antibodies.

Competing interests

The authors declare that they have no competing interests.

Access to questionnaires

For access to the questionnaires, visit www.healthpsychologyresearch.com.

Authors' contributions

EQ initiated and coordinated the linguistic validation, designed both studies, collected and analysed the data and wrote the manuscript. AV initiated the overall project. JK supervised laboratory analyses. SK recruited patients and supervised data analysis and drafting of the manuscript. All authors read and approved the final manuscript.

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References

1. Vanderpump M: **The epidemiology of thyroid disease.** *Br Med Bull* 2011, **99**:39–51.
2. Blondi B, Wartofsky L: **Combination treatment with T4 and T3: toward personalized replacement therapy in hypothyroidism?** *J Clin Endocrinol Metab* 2012, **97**(7):2256–2271.
3. Wartofsky L, Dickey RA: **The evidence for a narrower thyrotropin reference range is compelling.** *J Clin Endocrinol Metab* 2005, **90**(9):5483–5488.
4. Kratzsch J, Schubert G, Pulzer F, Pfaeffle F, Pfaeffle R, Koerner A, Dietz A, Rauh M, Kiess W, Thiery J: **Reference intervals for TSH and thyroid hormones are mainly affected by age, body mass index and number of blood leucocytes, but hardly by gender and thyroid autoantibodies during the first decade of life.** *Clin Biochem* 2008, **41**:1091–1098.
5. Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, Pessah-Pollack R, Singer PA, Woeber KA: **Clinical practice guidelines for hypothyroidism in adults: cosponsored by the american association of clinical endocrinologists and the american thyroid association.** *Endocr Pract* 2012, **18**(6):988–1028.
6. Saravanan P, Chau W-F, Roberts N, Vedhara K, Greenwood R, Dayan CM: **Psychological well-being in patients on 'adequate' doses of l-thyroxine: results of a large, controlled community-bases questionnaire study.** *Clin Endocrinol* 2002, **57**:577–585.
7. Samuels MH, Schuff KG, Carlson NE, Carello P, Janowsky JS: **Health status, psychological symptoms, mood, and cognition in l-thyroxine-treated hypothyroid subjects.** *Thyroid* 2007, **17**(3):249–258.
8. Walsh JP: **Dissatisfaction with thyroxine therapy – could the patients be right?** *Curr Opin Pharmacol* 2002, **2**:717–722.
9. Carta MG, Loviselli A, Hardoy MC, Massa S, Cadeddu M, Sardu C, Carpiniello B, Dell'Osso L, Mariotti S: **The link between thyroid autoimmunity (antithyroid peroxidase autoantibodies) with anxiety and mood disorders in the community: a field of interest for public health in the future.** *BMC Psychiatry* 2004, **4**:25.
10. Ott J, Promberger R, Kober F, Neuhold N, Tea M, Huber JC, Hermann M: **Hashimoto's thyroiditis affects symptom load and quality of life unrelated to hypothyroidism: a prospective case–control study in women undergoing thyroidectomy for benign goiter.** *Thyroid* 2011, **21**(2):161–167.
11. Groer MW, Vaughan JH: **Positive thyroid peroxidase antibody titer is associated with dysphoric moods during pregnancy and postpartum.** *J Obstet Gynecol Neonatal Nurs* 2013, **42**(1):E26–E32.
12. Panicker V, Saravanan P, Vaidya B, Evans J, Hattersley AT, Frayling TM, Dayan C: **Common variation in the DIO2 gene predicts baseline psychological well-being and response to combination of thyroxine plus triiodothyronine therapy in hypothyroid patients.** *J Clin Endocrinol Metab* 2009, **94**:1623–1629.
13. Kong WM, Sheikh MH, Lumb PJ, Freedman DB, Crook M, Doré CJ, Finer N: **A 6-month randomized trial of thyroxine treatment in women with mild subclinical hypothyroidism.** *Am J Med* 2002, **112**:348–354.
14. Ladenson PW: **Psychological wellbeing in patients.** *Clin Endocrinol* 2002, **57**:575–576.
15. Bradley C: **Importance of differentiating health status from quality of life.** *Lancet* 2001, **357**:7–8.
16. Razvi S, McMillan C, Weaver JU: **Instruments used in measuring symptoms, health status and quality of life in hypothyroidism: a systematic review.** *Clin Endocrinol* 2005, **63**:617–624.
17. Bell RJ, Rivera-Woll L, Davison SL, Topliss DJ, Donath S, Davis SR: **Well-being, health-related quality of life and cardiovascular disease risk profile in**

women with subclinical thyroid disease – a community based study. *Clin Endocrinol* 2007, **66**:548–556.
18. de Vries M, Ouwendijk R, Kessels AG, de Haan MW, Flobbe K, Hunink MGM, van Engelshoven JMA, Nelemans PJ: **Comparison of generic and disease-specific questionnaires for the assessment of quality of life in patients with peripheral arterial disease.** *J Vasc Surg* 2005, **41**:261–268.
19. Eurich DT, Johnson JA, Reid KJ, Spertus JA: **Assessing responsiveness of generic and specific health related quality of life measures in heart failure.** *Health Qual Life Outcomes* 2006, **4**:89.
20. Bianchi GP, Zaccheroni V, Solaroli E, Vescini F, Cerutti R, Zoli M, Marchesini G: **Health-related quality of life in patients with thyroid disorders.** *Qual Life Res* 2004, **13**:45–54.
21. Grabe HJ, Völzke H, Lüdemann J, Wolff B, Schwahn C, John U, Meng W, Freyberger HJ: **Mental and physical complaints in thyroid disorders in the general population.** *Acta Psychiatr Scand* 2005, **112**:286–293.
22. Tagay S, Herpertz S, Langkafel M, Erim Y, Freudenberg L, Schöpper N, Bockisch A, Senf W, Görges R: **Health-related quality of life, anxiety and depression in thyroid cancer patients under short-term hypothyroidism and TSH-suppressive levothyroxine treatment.** *Eur J Endocrinol* 2005, **153**:755–763.
23. Bauer M, Silverman HS, Schlagenhauf F, London ED, Geist CL, van Herle K, Rasgon N, Martinez D, Miller K, van Herle A, Berman SM, Phelps ME, Whybrow PC: **Brain glucose metabolism in hypothyroidism: a positron emission tomography study before and after thyroid hormone replacement therapy.** *J Clin Endocrinol Metab* 2009, **94**:2922–2929.
24. McMillan C, Bradley C, Woodcock A, Razvi S, Weaver JU: **Design of new questionnaires to measure quality of life and treatment satisfaction in hypothyroidism.** *Thyroid* 2004, **14**(11):916–925.
25. McMillan C, Bradley C, Razvi S, Weaver J: **Evaluation of new measures of the impact of hypothyroidism on quality of life and symptoms: the ThyDQoL and ThySRQ.** *Value Health* 2008, 11(2):285–294.
26. McMillan C, Bradley C, Razvi S, Weaver J: **Psychometric evaluation of a new questionnaire measuring treatment satisfaction in hypothyroidism: the ThyTSQ.** *Value Health* 2006, **9**(2):132–139.
27. Gärtner R, Gasnier BCH, Dietrich JW, Krebs B, Angstwurm WA: **Selenium supplementation in patients with autoimmune thyroiditis decreases thyroid peroxidase antibodies concentrations.** *J Clin Endocrinol Metab* 2002, **87**:1687–1691.
28. Gärtner R, Reincke M: **Substitution von Schilddrüsenhormonen.** *Internist* 2008, **49**(5):538–544.
29. Riazi A, Bradley C, Barendse S, Ishii H: **Development of the well-being questionnaire short-form in Japanese: the W-BQ12.** *Health Qual Life Outcomes* 2006, **4**:40.
30. Bullinger M, Kirchberger I: *SF-36. Fragebogen zum Gesundheitszustand Handanweisung.* Göttingen: Hogrefe; 1998.
31. Franke GH: *SCL-90-R Symptomcheckliste von L.R. Derogatis – Deutsche Version, 2. Auflage.* Göttingen: Beltz Test GmbH; 2002.
32. Witthaus E, Stewart J, Bradley C: **Treatment satisfaction and psychological well-being with insulin glargine compared with NPH in patients with Type 1 diabetes.** *Diabet Med* 2001, **18**(8):619–625.
33. Hautzinger M, Bailer M, Worall H, Keller F: *Beck-Depressions-Inventar (BDI).* Bern: Hans Huber Verlag; 1994.
34. Baumann U: **Methodische Untersuchungen zur Hamilton-Depressionsskala.** *Arch Psychiatr Nervenkr* 1976, **222**:359–375.
35. Brabant G, Beck-Peccoz P, Jarzab B, Laurberg P, Orgiazzi J, Szabolcs I, Weetman AP, Wiersinga WM: **Is there a need to redefine the upper normal limit of TSH?** *Eur J Endocrinol* 2006, **154**:633–637.
36. Vanderpump M: **Subclinical hypothyroidism: the case against treatment.** *Trends Endocrinol Metab* 2003, **14**(6):262–266.
37. Biondi B, Cooper DS: **The clinical significance of subclinical thyroid dysfunction.** *Endocr Rev* 2008, **29**(1):76–131.

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Structural and functional MRI study of the brain, cognition and mood in long-term adequately treated Hashimoto's thyroiditis

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Mood

Abstract The current study investigated neuropsychological and underlying structural and functional brain alterations in long-term adequately treated patients with Hashimoto's thyroiditis in order to examine much discussed residual complaints in patients in relation to possible long-term neural alterations with a specific interest in the underlying autoimmune process.

Eighteen patients with treated hypothyroidism due to Hashimoto's thyroiditis (mean age 32, range 18—54 years; two males; mean treatment duration 4.4 years) and 18 healthy matched control subjects underwent 3-Tesla magnetic resonance imaging (MRI). Voxel-based morphometry was used to investigate grey matter density, resting-state functional MRI to analyse the brain connectivity of areas known to be altered in hypothyroidism and event-related functional MRI to examine brain activity during associative memory encoding. Neuropsychological assessment included memory, working memory, psychomotor speed and attention. We previously reported subclinically reduced mood in this study population and investigated its neural correlates here. Thyroid stimulating hormone, free triiodthyronine, free thyroxine and thyroid peroxidase antibodies were measured in serum.

We did not find cognitive deficits or alterations in grey matter density, functional connectivity or associative memory-related brain activity in comparison to the control group and cognition was unrelated to thyroid serum measures in the patient group. Thyroid peroxidase antibodies in the patient group correlated with increased grey matter density in right amygdala and enhanced

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connectivity between subcallosal and parahippocampal areas. Treatment duration was associated with brain structure in frontal and occipital cortex and connectivity between left amygdala and frontal cortex. Mood correlated with brain areas associated with distinct functional networks, but not with those most prominently affected in depression.

In conclusion, no cognitive or neural alterations were detected in this young and otherwise healthy cohort of patients in comparison to a healthy control group and current mood status could not be related to depression-related networks. However, autoimmune activity and treatment duration showed a relationship with depression and hypothyroidism-related brain structure and function. They are thus promising factors to further investigate residual complaints despite biochemically adequate treatment in patients with Hashimoto's thyroiditis. Given the small sample size, all findings require replication.

1. Introduction

Hypothyroidism or subclinical hypothyroidism affect about 8% of the female and 3% of the male population. Standard treatment is replacement of thyroid hormone by levothyroxine, artificial free thyroxine. However, it has been reported that among patients receiving biochemically adequate treatment, well-being (Saravanan et al., 2002; Wekking et al., 2005) and cognitive performance remain reduced, although reports about cognition are less consistent with some studies reporting residual deficits (Wekking et al., 2005; Correia et al., 2009) and others not (Miller et al., 2006). It is still an open issue where residual alterations result from. Explanations discussed are insufficient normalisation of thyroid hormone levels at target tissues such as the brain despite normal serum hormone levels (Panicker et al., 2009), independent effects of thyroid autoimmunity by thyroid peroxidase antibodies (TPO-ab), the most common cause of hypothyroidism (Ott et al., 2011), selection bias in seeking health care (Kong et al., 2002) and reactive processes to the awareness of having a chronic disease (Ladenson, 2002). Disentangling these possible causes has important implications for treatment strategies. The current study wants to contribute to the discussion by studying the target organ of interest, the brain, and individual TPO-ab activity.

Changes in brain metabolism and regional cerebral blood flow measured by positron-emission tomography (PET) and single photon-emission computed tomography (SPECT) have reliably been reported in hypothyroidism and proved reversible in some studies (Bauer et al., 2009; Schraml and Beason-Held, 2010), whereas others showed persevering alterations after short-term treatment (Krausz et al., 2004; Nagamachi et al., 2004). In terms of localisation, areas across all four brain lobules have been reported (Nagamachi et al., 2004), also in a recent structural magnetic resonance imaging (MRI) study on untreated patients (Singh et al., 2013) using voxel-based-morphometry (VBM), a method allowing voxel-wise comparison of grey matter density (Ashburner and Friston, 2000). One PET study was able to show a direct relationship between the restoration of brain metabolism with treatment and the normalisation of thyroid stimulating hormone (TSH), symptom load and mood in anterior and posterior cingulate cortex, amygdala and hippocampus (Bauer et al., 2009). These areas therefore are most likely to help us understand residual complaints in long-term treated patients. Task-based functional MRI (fMRI) studies in hypothyroidism have focused on working memory and have shown fully reversible alterations in attentional load effects in frontoparietal areas (Zhu et al., 2006). No brain imaging studies are known to us on other cognitive functions discussed in adult-onset hypothyroidism such as associative memory, although animal research suggests hippocampal involvement (Alzoubi et al., 2009) and altered hippocampal activity during associative memory has been shown in teenagers with congenital hypothyroidism (Wheeler et al., 2011). A behavioural study found impaired associative encoding also in adult-onset short-term treated hypothyroidism (Correia et al., 2009). Resting-state fMRI is a relatively new method that allows the investigation of neural activity between brain areas during rest, representing an important feature of the brain, organisation in functional networks. It thus ideally supplements structural MRI methods such as VBM that are confined to the study of brain regions in isolation (Fox and Raichle, 2007). We used resting-state fMRI to investigate functional networks of areas known to be involved in hypothyroidism (Bauer et al., 2009) and to explore the neural correlates of mood.

Brain imaging studies have mainly focused on untreated or short-term treated hypothyroidism and effects of long-term hormone supplementation are little known. Only two studies examined longer-term treated patients, both focusing on autoimmune thyroid disease. The first found diffuse hypoperfusion using SPECT (Zettinig et al., 2003), the other only reported a very specific correlation between grey matter density in left inferior frontal gyrus and attention using VBM (Leyhe et al., 2013). More data is thus needed on long-term treated Hashimoto's disease, which is of great clinical importance because it is a chronic condition requiring lifelong hormone substitution and thus patient numbers are large.

Another focus of the study is the autoimmune activity ongoing in Hashimoto's thyroiditis independent of the hormone substitution. It has been shown that patients with adequately treated Hashimoto's disease (Ott et al., 2011) and antibody positive members of the general population (Grabe et al., 2005) have a heightened risk for depression and anxiety. A placebo-controlled study showed that TPO-ab activity can be reduced by selenium supplementation alongside subjective health improvement (Gärtner et al., 2002). Brain imaging studies on patients with Hashimoto's thyroiditis are limited to three SPECT studies showing diffuse hypoperfusion across treatment and thyroid hormone status (Zettinig et al., 2003; Piga et al., 2004; Kaya et al., 2007) and the single VBM study mentioned above (Leyhe et al., 2013). The data

suggest brain alterations in Hashimoto's disease, but data on other brain imaging modalities are lacking.

We have thus included 18 currently euthyroid patients on stable doses of levothyroxine for hypothyroidism due to autoimmune thyroiditis (Hashimoto's disease), and 18 healthy matched control subjects. Brain MRI included structural (VBM) as well as resting-state and event-related fMRI in order to investigate grey matter structure, functional connectivity and brain activity during associative memory encoding. The study design included laboratory assessment of TSH, TPO-ab, free triiodothyronine (fT3) and free thyroxine (fT4). Moreover, we used mood parameters from Quinque et al. (2013) for correlation analyses and performed a high-load cognitive test battery to detect subtle deficits in functions discussed in hypothyroidism: memory, working memory, psychomotor speed and attention (Wekking et al., 2005). In order to reduce noise within neuropsychological, laboratory and MRI data, we performed all measurements twice and averaged the data (Van Dijk et al., 2010). In addition to comparing the two study groups, we correlated laboratory measures with cognition and MRI parameters in the patient group to investigate associations between current biochemical thyroid status, most importantly TPO-ab activity and cognition, brain structure and function. Moreover, mere effects of duration of treatment on the above parameters were analysed. In summary, the design allowed investigation of residual behavioural and neural alterations in long-term adequately treated patients with Hashimoto's thyroiditis.

2. Methods

2.1. Sample and procedure

Twenty-five patients with levothyroxine treated hypothyroidism due to autoimmune thyroiditis and 27 healthy control subjects were enrolled and reimbursed for their time. Patients were recruited via internet advertisement, local endocrinologists and the institute's volunteer database, healthy control subjects via the database alone. Two patients did not complete the study for reasons unrelated to disease status. Five patients and nine healthy control subjects had to be excluded due to unstable doses or thyroid laboratory values outside the reference range. The research protocol was approved by the ethics committee of the University of Leipzig and was in accordance with the latest version of the Declaration of Helsinki. All participants took part in five study visits, first, an information session to obtain written informed consent, second, a neuropsychological test session of 2.5 h and thirdly an MRI session of 1.5 h including blood withdrawal. The second and third visits were repeated in identical form after three to six months. Neuropsychological testing and MRI session were generally performed within one week and groups did not differ in the mean interval. For organisational reasons, 9 of the 72 analysed session pairs had longer intervals between 9 and 39 days.

2.2. Neuropsychological data collection

Participants reported basic sociodemographic data and filled in a series of patient-reported outcome measures as reported in Quinque et al. (2013) and performed a neuropsychological test battery. The tasks were selected to cover all functions discussed in hypothyroidism at a high-load level in order to detect possibly subtle deficits. Testing included intelligence screening (Schmidt and Metzler, 1992) for matching purposes, tests on verbal memory (word list learning: California Verbal Learning Test, Niemann et al., 2008 and story recall: Wechsler-memory scale, WMS logical memory, Wechsler, 1987), working memory span (WMS digit and block span), attentional speed (Testbatterie zur Aufmerksamkeitsprüfung, TAP reaction times of subtests alertness, working memory and divided attention, Zimmermann and Fimm, 2007 and of Trail-Making-Test, TMT, Reitan and Wolfson, 1993), sustained attention (Paced Auditory Serial Addition Test, PASAT, Gronwall, 1977, TAP working memory errors and misses) and attention lapses (TAP misses of subtests alertness and divided attention, TMT errors).

2.3. MRI data acquisition

MRI was performed on a 3-Tesla MAGNETOM Verio scanner (Siemens, Erlangen, Germany) equipped with a 32-channel head array coil. We acquired T1-weighted images as well as resting-state and event-related fMRI. During the latter participants performed an associative memory encoding task adapted from Sperling et al. (2003), see Fig. 1. FLAIR images were acquired for neurological screening of the subjects. Total time in the scanner was 60 min per visit, see supplemental material for acquisition details.

2.4. Blood sample

Fasting blood samples were taken in the morning between 6.45am and 10am directly before the MRI scan to assess levels of TSH, fT3, fT4, TPO-ab and thyroglobulin antibodies, the latter only for ensuring thyroid health of the control group. Patients refrained from taking their medication before the blood withdrawal. Blood samples were stored at −80 °C and analysed at the Institute for Laboratory Medicine of the University Hospital Leipzig by the fully automated Roche cobas electrochemiluminescence system (Roche, Basel, Switzerland). The sensitivity of the assays was 0.005 mIU/l, 0.4 pmol/l, 0.3 pmol/l and 5 IU/ml for TSH, fT3, fT4 and TPO-ab. The measurements were done during clinical routine analysis in parallel with the analysis of quality control samples. The intra- and interassay coefficients of variation for quality control samples ($n > 117$) were exemplified <3.2% for TSH (1.64 and 8.39 mIU/l), <3.3% for fT3 (5.64 and 23.5 pmol/l), <4.8% for fT4 (16.0 and 48.0 pmol/l) and <10.3% for TPO-ab (38.1 and 108.2 IU/ml) for this time frame.

2.5. General data analysis

All data were averaged over the two test sessions to increase sensitivity by reducing noise (Van Dijk et al., 2010) as no systematic changes were expected for any of the two groups investigated for any of the parameters. Chi-square tests were used for comparison of nominal data, t-tests or analyses of covariance (ANCOVAs) for group comparisons and Pearson correlations for correlating biochemical thyroid status with cognition and MRI data in the patient group. All correlation

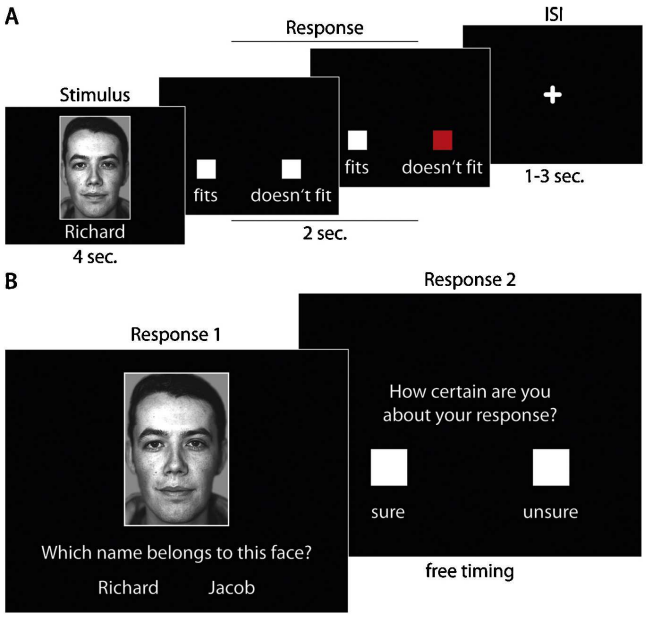

Fig. 1 Example trial of (A) the associative memory encoding task performed during event-related functional magnetic resonance imaging and (B) the offline retrieval task performed in the scanner directly after the functional scan. Abbreviations: ISI—inter-stimulus-interval.

analyses with MRI data were corrected for age and sex. Statistical significance for behavioural data was accepted if $p < 0.05$ and for MRI data if $p < 0.001$ at voxel level and $p < 0.05$ at family-wise error corrected cluster size level. Bonferroni correction for multiple testing was applied where necessary.

Those three mood parameters that showed significant impairment in the treated patient group as reported in Quinque et al. (2013) were used for correlation analyses with MRI data in the patient group. These were depression measured by the Beck Depression Inventory (Hautzinger et al., 1994), disease-specific symptom load measured by the hypothyroidism symptom questionnaire (ThySRQ, McMillan et al., 2008) and well-being measured by the generic well-being questionnaire-12 (Witthaus et al., 2001). Finally, treatment duration was correlated with MRI data in the patient group to investigate possible influences of the duration of medication, an approximation of the duration of the disease, on brain structure and function.

2.6. Neuropsychological data analysis

SPSS statistics version 19 was used for analyses. ANCOVAs were used to compare the two groups. For the correlation analysis individual task results were z-transformed and averaged into summary scores representing the four cognitive domains memory, working memory, psychomotor speed and sustained attention, see Table 2. The standardised summary scores were then correlated with biochemical thyroid status.

2.7. MRI data analysis

T1-weighted images were preprocessed using standard parameters of the VBM8 toolbox for longitudinal data (http://dbm.neuro.uni-jena.de/vbm.html) for SPM8 (Wellcome Trust Centre for Neuroimaging, University College London, London, UK; http://www.fil.ion.ucl.ac.uk/spm), running on Matlab version 7.11 (Mathworks, Cambridge, UK; http://www.mathworks.com) using methods described in Ashburner and Friston (2005), see supplemental material. In order to increase sensitivity to regions shown to be affected in hypothyroid patients, the group analyses were done whole brain and in a region of interest (ROI) approach using six regions derived from Bauer et al. (2009) including left and right hippocampus, left and right amygdalae, posterior cingulated cortex and subcalossal cortex, see supplemental material. All analyses were additionally controlled for total intracranial volume and nonstationary cluster correction was applied (Hayasaka et al., 2004).

Image preprocessing of the resting-state data was performed with Freesurfer, Functional magnetic resonance imaging of the brain Software Library (FSL) and Analysis of Functional NeuroImages (AFNI) standard scripts distributed by 1000 Functional Connectome Project (http://fcon_1000.-projects.nitrc.org) run on SPM8 and in-house scripts written in Matlab. The same six regions used as ROIs for the VBM analysis were entered as seed regions. An additional functional connectivity analysis was performed with those areas as seeds that revealed a significant correlation between grey

matter density and mood in the patient group (see Supplemental material).

Task-based fMRI data was preprocessed with standard procedures, see supplemental material. Trials of the associative memory task were classified into remembered trials defined as correctly identified at retrieval with subjective certainty, all others were classified as forgotten. In addition, the overall percentage of correctly identified trials was registered. Behavioural performance was compared between groups. For each participant two first-level maps were generated, one contrasting nullevents to all encoding trials (ALL_NULL contrast), the other encoding trials of subsequently remembered trials to those that were forgotten (REM_FOR contrast). Onesample *t*-tests were performed for both groups separately in order to investigate the general task network.

3. Results

3.1. Sample

In the final sample, 18 currently euthyroid treated patients with hypothyroidism due to proven autoimmune thyroiditis and stable levothyroxine doses and 18 healthy matched control subjects were included. All participants were free of other chronic diseases and lifetime psychiatric or neurological illnesses. All included control subjects were thyroid healthy and TPO-ab and thyroglobulin antibody negative. Samples were matched for age, sex, intelligence and TSH, see Table 1. Groups were additionally matched for handedness (one left per group), smoking status (three smokers per group) and use of estrogen containing contraceptives (five patients, six control subjects). All subsequent analyses were controlled for age, sex, TSH, fT3 and fT4 to ensure ideal matching for biochemical thyroid status (Samuels et al., 2007). Analyses were repeated with control for age and sex alone to investigate group differences under the influence of differences in biochemical status between treated and healthy participants.

3.2. Neuropsychology

Results of the high-load neuropsychological test battery are summarised in Table 2. The single significant group difference showed better learning performance in the patient than the control group, but would not survive multiple comparison correction. Furthermore, none of the domain summary scores was significant and there was no descriptive trend in favour of any of the two groups investigated. Without control for differences in biochemical thyroid status again no significant group differences emerged that would survive Bonferroni correction. Attention lapses were generally very rare, see Table 2, so that the data were not used for further correlation analyses.

Correlation between the four cognitive domains investigated and biochemical thyroid status in the patient group revealed a significant correlation between fT3 and psychomotor speed that would however not survive correction for multiple comparisons, see Table 3 for exact results. Explorative inspection of the whole correlation table revealed only small or inconsistent relationships between current biochemical thyroid status and cognition.

3.3. Voxel-based morphometry

There were no significant differences in grey matter density between the two groups. Increasing the sensitivity of the analysis by means of an ROI analysis reducing the analysis to the six areas identified to be related to hypothyroidism by Bauer et al. (2009) also revealed no significant group

Table 1 Characteristics of the clinical sample with adequately treated hypothyroidism in proven autoimmune thyroid disease and the healthy control group.

Sample characteristics	Treated patient group $n = 18$			Healthy control group $n = 18$			p-Value
	Mean	SD	Range	Mean	SD	Range	
Age (y)	32	9.6	18–54	32	8.9	18–53	>0.1
Intelligence (IQ)	107	7.9	95–122	108	9.3	93–125	>0.1
Sex (female/male)		16/2			16/2		>0.1
TSH (mU/l)*	2.0	1.1	0.4–4.1	2.5	0.7	0.9–3.6	>0.1
fT3 (pmol/l)*	4.3	0.6	3.3–5.5	4.7	0.7	3.4–6.0	<0.5
fT4 (pmol/l)*	18	1.6	14–21	16	2.2	14–23	<0.001
TPO-ab (U/ml)*	140	132	9–457	10	2.1	7–14	<0.001
Pulse (beats/min)**	71	7.1	58–82	70	8.2	56–86	>0.1
Systolic BP***	118	13	96–133	116	9.1	106–139	>0.1
Diastolic BP***	77	9.6	62–84	74	4.7	66–80	>0.1
Treatment duration (y)	4.4	4.2	1–15	not applicable			
Current dose (μg/day)	97	39	50–150	not applicable			

* Reference range was 0.4–4.0 for thyroid stimulating hormone (TSH), 3.1–6.79 for free triiodothyronine (fT3), 12.8–20.4 for free thyroxine (fT4), <34 for thyroid peroxidase antibodies (TPO-ab) (Kratzsch et al., 2008). Patients for whom laboratory values exceeded the reference range only in one of the two sessions were considered as thyroid healthy or adequately treated and included into the analysis.

** Only available in 17 patients and 15 control subjects.

*** Only available in 15 control subjects. Abbreviations: SD—standard deviation, y—years, IQ—intelligence quotient with population mean = 100, SD = 15, min—minutes, BP—blood pressure.

Table 2 Raw scores and derived domain z-scores of the neuropsychological test battery and results of group comparisons by means of analyses of covariance controlling for age, TSH, fT3 and fT4 (model 1) or for age alone (model 2).

	Treated patient group $n = 18$	Healthy control group $n = 18$	p-Value model 1	p-Value model 2
Verbal memory	0.03 ± 0.7	-0.03 ± 0.9	>0.1	>0.1
CVLT immediate recall A1	8.75 ± 2	9.06 ± 2	>0.1	>0.1
CVLT learning A5	15.0 ± 1	14.3 ± 1	=0.034	>0.1
CVLT inference list B	6.61 ± 2	6.36 ± 1	>0.1	>0.1
CVLT short delay free recall	14.2 ± 2	13.8 ± 2	>0.1	>0.1
CVLT long delay free recall	14.3 ± 2	13.8 ± 2	>0.1	>0.1
WMS immediate story recall	34.1 ± 5	35.9 ± 5	>0.1	>0.1
WMS delayed story recall	31.5 ± 5	32.9 ± 7	>0.1	>0.1
Working memory span	-0.20 ± 0.6	0.21 ± 0.8	>0.1	>0.05
WMS Digit span forward	6.18 ± 1	6.44 ± 1	>0.1	>0.1
WMS Digit span backward	4.82 ± 1	4.78 ± 1	>0.1	>0.1
WMS Block span forward	5.50 ± 1	6.03 ± 1	>0.1	>.05
WMS Block span backward	5.26 ± 1	5.90 ± 1	>0.1	=.007
Psychomotor speed	0.09 ± 0.5	-0.09 ± 0.7	>0.1	>0.1
TAP alertness tonic RT (ms)	234 ± 28	232 ± 32	>0.1	>0.1
TAP alertness phasic RT (ms)	230 ± 26	230 ± 32	>0.1	>0.1
TAP working memory RT (ms)	584 ± 122	595 ± 106	>0.1	>0.1
TAP divided auditory RT (ms)	568 ± 83	542 ± 66	>0.1	>0.1
TAP divided visual RT (ms)	760 ± 55	735 ± 45	>0.1	>0.1
TMT A time (s)	20.9 ± 5	19.6 ± 5	>0.1	>0.1
TMT B time (s)	47.1 ± 12	45.0 ± 13	>0.1	>0.1
Sustained attention	-0.06 ± 0.8	0.06 ± 0.7	>0.1	>0.1
PASAT 2.4 errors	6.47 ± 7	5.22 ± 4	>0.1	>0.1
PASAT 1.6 errors	11.5 ± 7	9.86 ± 7	>0.1	>0.1
TAP working memory errors	1.36 ± 3	1.94 ± 2	>0.1	>0.1
TAP working memory misses	0.86 ± 1	1.61 ± 1	>0.1	>.05
Attention lapses	0.10 ± 0.5	-0.10 ± 0.4	>0.1	>0.1
TAP alertness tonic misses	0.08 ± 0.3	0.03 ± 0.1	>0.1	>0.1
vTAP alertness phasic misses	0.06 ± 0.2	0.03 ± 0.1	>0.1	>0.1
TAP divided auditory misses	0.39 ± 0.7	0.03 ± 0.1	>0.1	>.05
TAP divided visual misses	0.64 ± 0.7	0.36 ± 0.6	>0.1	>0.1
TMT A errors	0.19 ± 0.3	0.25 ± 0.5	>0.1	>0.1
TMT B errors	0.31 ± 0.3	0.42 ± 0.6	>0.1	>0.1

Abbreviations: CVLT—California Verbal Learning Test, WMS—Wechsler memory scale, TAP—Testbatterie zur Aufmerksamkeitsprüfung, RT—reaction time, TMT—Trail-Making-Test, PASAT—paced auditory serial addition test.

differences at the corrected significance level ($p < 0.0083$). The same results were found when controlling for age and sex alone.

Correlation analysis between grey matter density and biochemical thyroid status revealed that TSH was correlated with reduced grey matter density in left anterior cingulate cortex ($p = 0.007$, peak voxel at Montreal Neurological Institute (MNI) coordinates $x = -8$ $y = 42$ $z = 3$, cluster size 103 voxels) and TPO-ab with increased grey matter density in right amygdala ($p = 0.031$, peak voxel 33 2 −26, 52 voxels). Correlation with mood parameters revealed a positive correlation between depression and grey matter density in right postcentral gyrus ($p = 0.007$, peak voxel 3 −30 51, 521 voxels), left superior frontal gyrus ($p < 0.001$, peak voxel −6 24 58, 323 voxels) and left cuneus ($p = 0.038$, peak voxel −4 −76 34, 279 voxels) and a negative correlation with grey matter density in left middle temporal gyrus ($p = 0.004$, peak voxel −60 −7 −27, 290 voxels). In addition well-being was negatively correlated to grey matter density in left temporal pole ($p = 0.005$, peak voxel −44 5 −24, 103 voxels) see Fig. 2. The explorative functional connectivity analysis revealed several distinct networks (see Supplemental material results and Fig. S1).

Treatment duration in the patient group was positively correlated with right supracalcarine cortex ($p = 0.046$, peak voxel 3 −84 9, 76 voxels) and negatively with right inferior frontal gyrus ($p = 0.049$, peak voxel 56 24 1, 60 voxels).

3.4. Resting-state functional connectivity

Groups did not differ in offline-assessed pulse or blood pressure (see Table 1), so that important confounders of resting-state activity are unlikely to account for the current results. Resting-state functional networks of six regions identified to be altered in untreated hypothyroidism (Bauer et al., 2009) were not significantly different between groups, even at an uncorrected significance level. The same results were found when controlling for age and sex alone. Correlation analysis of the connectivity maps with biochemical thyroid status showed a correlation between fT4 and reduced connectivity strength between left amygdala and right

Table 3 Pearson correlations between biochemical thyroid parameters and cognitive domains in the patient group.

	TSH	fT3	fT4	TPO-ab
Verbal memory	0.08	−0.21	−0.10	0.01
Working memory span	−0.25 [1]	0.12 [1]	0.11 [1]	−0.02 [1]
Psychomotor speed	−0.42	−0.54 [1*]	−0.41 [1]	−0.26
Sustained attention	0.17 [1]	−0.19 [1]	−0.28 [1]	0.07 [1]

* $p = 0.022$.
[1] In the expected direction; abbreviations: TSH—thyroid stimulating hormone, fT3—free triiodothyronine, fT4—free thyroxine, TPO-ab—thyroid peroxidase antibodies.

middle temporal gyrus ($p = 0.002$, peak voxel 54 −21 −9, 70 voxels) as well as between subcallosal cortex and right frontal pole ($p = 0.004$, peak voxel 21 51 0, 68 voxels). TPO-ab levels were correlated with increased connectivity between subcallosal cortex and left parahippocampal gyrus ($p = 0.002$, peak voxel −39 −15 −18, 72 voxels). Mood parameters did not correlate with functional connectivity even at an uncorrected cluster size level of $p < 0.05$, but treatment duration correlated significantly with increased connectivity between left amygdala and two frontal regions (right superior $p = 0.008$, peak voxel 6 54 39, 54 voxels, right middle $p = 0.002$, peak voxel 36 0 42, 73 voxels), see Fig. 2.

3.5. Task-based fMRI

Patients remembered the association between name and photograph in 49% of the trials (SD = 14) and control subjects in 40% of the trials (SD = 13). Overall percentage of correct trials was 74% (SD = 8) in the patient group and 67% (SD = 9) in the control group. There were no significant group differences for number of remembered trials ($F(1,30) = 2.75$; $p > 0.1$) or percentage correct ($F(1,30) = 0.53$; $p > 0.1$) in the main analysis, but if controlling for age alone there was a trend for more remembered trials in the patient group ($F(1,33) = 3.92$; $p = 0.056$) and percentage correct even reached significance ($F(1,33) = 6.14$; $p = 0.019$). In order to

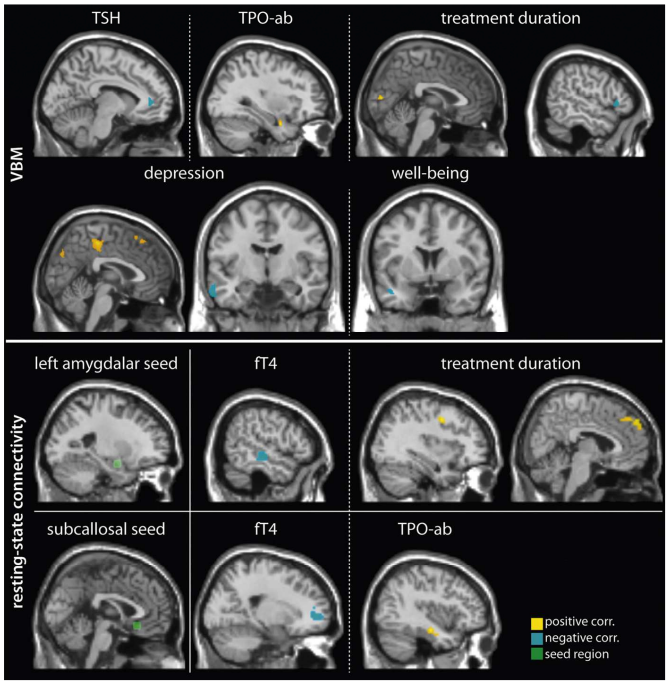

Fig. 2 Significant correlations between biochemical thyroid status, mood, treatment duration and brain structure (VBM) and function (resting-state connectivity) at MNI coordinates of the respective peak voxel. Images are presented in sagittal or coronal view oriented with neurological convention. Abbreviations: VBM—voxel-based morphometry, MNI—Montreal Neurological Institute, TSH—thyroid stimulating hormone, TPO-ab—thyroid peroxidase antibodies, fT4—free thyroxine, corr—correlations.

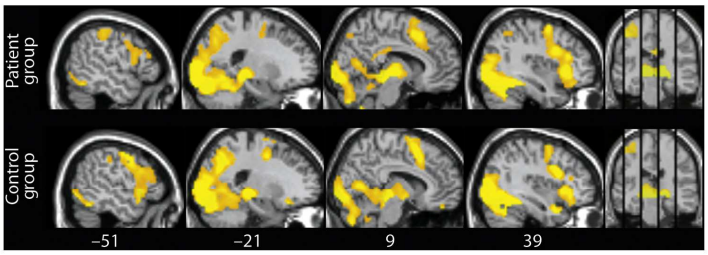

Fig. 3 Task network of the associative memory encoding paradigm in both groups, results of onesample *t*-tests controlled for age and sex are shown on sagittal brain slices oriented with neurological convention.

remove any influence of task performance on MRI results, the subsequent analyses were corrected for percentage correct.

Contrasting all trials to baseline revealed a widespread task positive network in both groups including bilateral occipital and frontal areas, precentral gyri, fusiform cortices, hippocampi, thalami, posterior parietal areas and the cerebellum. This network included the expected areas for visual perception, face perception, associative learning and planning of finger movements (see Fig. 3).

No group difference was found in the task activity whole brain or within the ROIs associated with hypothyroidism, most notably not within hippocampus. The same results were found when controlling for age and sex alone. The control group showed significantly greater activity for remembered than forgotten trials in left temporal fusiform cortex ($p < 0.001$, peak voxel −42 −70 −5, 203 voxels) and right lateral occipital areas ($p < 0.001$, peak voxel 27 −82 19, 580 voxels) in a onesample *t*-test corrected for age and sex, that was not found in the patient group. However, direct comparison revealed no significant group difference. As the REM_FOR contrast was not found in the patient group, correlation analysis was only performed for the ALL_NULL contrast. The ALL_NULL contrast did not correlate with laboratory parameters, mood or treatment duration.

4. Discussion

There was no evidence for any cognitive deficit in long-term levothyroxine-treated patients with Hashimoto's thyroiditis neither in a high-load neuropsychological test battery covering memory, working memory, psychomotor speed, sustained attention and attention lapses nor in an associative memory task performed during fMRI acquisition. Even when refraining from ideal matching for biochemical thyroid status the groups did not differ. If any, we found a trend in favour of better performance in the patient group. Previous studies report data either in line with the current findings (Miller et al., 2006; Kramer et al., 2009) or showing residual impairment in distinct cognitive domains (Wekking et al., 2005; Samuels et al., 2007; Leyhe et al., 2008). It is possible that cognitive deficits only become apparent in patients with less cognitive reserve but not in an otherwise young, healthy and highly-educated population as studied here. The overall evidence concerning residual cognitive deficits in adequately treated patients remains, however, controversial and needs further investigation. In order to avoid biased conclusions, it is however of essential importance not to disregard the accumulating null findings.

We did not find significant correlations between cognition and biochemical thyroid status and no consistent pattern when exploring the full correlation table. This adds to a vast literature on the absence of an association between cognitive performance and thyroid hormone levels across patient and healthy populations and different age groups (Samuels et al., 2007; Booth et al., 2013; Castellano et al., 2013). In a carefully controlled intervention study, small levothyroxine dose changes resulting in altered TSH levels within the normal range did not influence cognitive performance (Walsh et al., 2006). Finally, it is important to note that fT4 and fT3 levels measured in the blood do not inevitably mirror their levels in the brain. T4 and T3 are taken up by astrocytes and T4 is subsequently converted to T3 by type-2 deiodinase (D2) enzyme and transported to neurons. Only T3 activates transcription of target genes in the neuron. Alterations in D2 enzyme activity would therefore lead to impaired conversion of T4 to T3 and thus contribute to alterations in neural function. However, the clinical significance of D2 alterations is still under investigation and should therefore be considered with caution (Fliers et al., 2006).

We did not find grey matter changes in long-term levothyroxine-treated Hashimoto's patients on a whole-brain level or in hypothesised regions of interest, similar to Leyhe et al. (2013). The second available VBM study on overtly hypothyroid patients found diffuse alterations (Singh et al., 2013), but so far there is no evidence for residual grey matter changes in adequately treated patients. We equally did not find any differences in functional connectivity between the two groups. As this was the first study employing resting-state fMRI in hypothyroid patients more studies are needed to further investigate functional connectivity alterations. It is still of relevance that we were unable to find alterations in networks of regions shown to be affected by hypothyroidism (Bauer et al., 2009). Finally, we did not find group differences in associative memory task-induced brain activity, most importantly not in the hippocampus. This is in line with research on hypothyroid rats, which showed hippocampal and memory alterations that were fully reversible with levothyroxine treatment (Alzoubi et al., 2009), but contrasts a study on adolescents with congenital hypothyroidism, which showed increased hippocampal activity during a memory task despite long-term adequate treatment (Wheeler et al., 2011).

Correlation results are necessarily preliminary due to the small sample size. Grey matter density in left subgenual anterior cingulate cortex (ACC) correlated with higher TSH in the treated patient group, a region identified to show reduced glucose metabolism during hypothyroidism (Bauer et al., 2009) and, in the contralateral analogue region, an association with change in TSH after treatment initiation. Higher fT4 in the patient group led to reduced resting-state functional connectivity between left amygdala and right middle temporal gyrus as well as between subcallosal cortex and right frontal pole, both part of the depression network (Quidé et al., 2012). This is in line with our own findings that showed a consistent association between higher fT4 and reduced depression scores (Quinque et al., 2013). TPO-ab levels were related to increased grey matter density in right amygdala and to increased connectivity between subcallosal cortex and parahippocampal gyrus, all related to the depression and fear network (Quidé et al., 2012). Given the reported increase in anxiety and depression in autoimmune thyroid disease (Grabe et al., 2005; Ott et al., 2011), this may have a specific neural correlate in the described regions. So far, only diffuse hypoperfusion was found in long-term adequately treated Hashimoto's thyroiditis (Zettinig et al., 2003), so that our more specific findings certainly need further exploration e.g. by experimentally manipulating TPO-ab activity by selenium supplementation (Gärtner et al., 2002).

Depression and well-being were related to grey matter density in several distributed areas that belonged to distinct networks (Beckmann et al., 2005; Fox and Raichle, 2007; Wang et al., 2012), but none to areas associated with affect such as the ACC, limbic structures or the medial frontal cortex (Quidé et al., 2012; Wang et al., 2012). These areas have been shown to be related with depression in untreated hypothyroid patients (Bauer et al., 2009; Schraml and Beason-Held, 2010), who showed higher depression scores than our sample, so that the relationship may only become apparent in more severely depressed patients. Moreover, we did not find correlations between mood and resting-state connectivity. It thus remains unclear where reported residual complaints result from (Saravanan et al., 2002; Wekking et al., 2005).

The duration of treatment was associated with an increase in grey matter in right supracalcarine cortex and a decrease in the right inferior frontal gyrus, both regions shown to be altered in hypothyroid patients (Krausz et al., 2004; Nagamachi et al., 2004; Singh et al., 2013). Connectivity strengths between left amygdala and right superior and middle frontal cortex increased with treatment duration, areas associated with depression and untreated hypothyroidism (Bauer et al., 2009; Schraml and Beason-Held, 2010; Singh et al., 2013). It may thus be hypothesised that transient hypothyroid states over the course of the progressive disease may result in chronic brain changes known from untreated hypothyroidism, but this idea needs further investigation by longitudinal studies covering a longer time span.

We acknowledge the small sample size of $n = 18$ per group. It is comparable to other clinical brain imaging studies (Bauer et al., 2009 $n = 13$; Singh et al., 2013 $n = 10$) in the field that were able to show differences in untreated hypothyroidism. However, as treated hypothyroidism is expected to produce less severe deficits we still cannot rule out that our sample size is too small to detect more subtle group differences and larger studies are needed before drawing any definite conclusions. Our clinical sample was of a young age range in order to exclude effects of aging and comorbidities on the data, and thus does not contribute to neural and cognitive effects of hypothyroidism in the elderly. Estrogen therapy has shown a differential age and disease status effect, in such that estrogen substitution is beneficial in healthy neurons but accelerates cell death due to oxidative stress in already diseased cells (Zhao et al., 2005). Similar age effects are likely for neural effects of thyroid hormone substitution. Brain imaging studies so far have concentrated on young to middle aged samples except for one population-based study that indeed shows an association between high normal fT4 levels and hippocampal atrophy (de Jong et al., 2006). Furthermore, cognitive functions show a curvilinear relationship to fT4 levels in the elderly (Hogervorst et al., 2008), suggesting that treatment should carefully be controlled in both directions in the elderly. Furthermore, we have only included subjects on adequate doses of levothyroxine, so that results only apply to patients currently meeting the reference range. Moreover, the majority of participants were female, owing to the gender bias in the prevalence of the disease, as a consequence of which our results can only be generalised reliably to the female population.

In conclusion, in a carefully controlled study design employing high-field structural and functional MRI as well as detailed high-load neuropsychological assessment, we were not able to show cognitive deficits or neural brain changes in long-term adequately treated patients with Hashimoto's thyroiditis in comparison to a healthy control group. Moreover, biochemical thyroid status was unrelated to cognitive performance. Given the small sample size we cannot completely rule out the possibility of a false negative finding. While we find indications for an association between mood-related brain areas and networks and both TPO autoimmunity and treatment duration and suggest them as promising lines of future research, larger datasets are necessary before drawing any definite conclusions. The search for neural correlates of current mood status revealed results that were less consistent with previous findings in depression and hypothyroidism. The search for neural correlates of residual complaints in adequately treated patients with Hashimoto's thyroiditis thus warrants further study.

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Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2014.01.015.

References

Alzoubi, K.H., Gerges, N.Z., Aleisa, A.M., Alkadhi, K.A., 2009. Levothyroxin restores hypothyroidism-induced impairment of hippocampus-dependent learning and memory: behavioral, electrophysiological, and molecular studies. Hippocampus 19, 66—78.

Ashburner, J., Friston, K.J., 2000. Voxel-based morphometry—the methods. NeuroImage 11, 805—821.

Ashburner, J., Friston, K.J., 2005. Unified segmentation. NeuroImage 26 (3) 839—851.

Bauer, M., Silverman, H.S., Schlagenhauf, F., London, E.D., Geist, C.L., van Herle, K., Rasgon, N., Martinez, D., Miller, K., van Herle, A., Berman, S.M., Phelps, M.E., Whybrow, P.C., 2009. Brain glucose metabolism in hypothyroidism: a positron emission tomography study before and after thyroid hormone replacement therapy. J. Clin. Endocrinol. Metab. 94, 2922—2929.

Beckmann, C.F., De Luca, M., Devlin, J.T., Smith, S.M., 2005. Investigations into resting-state connectivity using independent component analysis. Philos. Trans. R. Soc. London, Ser. B 360, 1001—1013.

Booth, T., Deary, I.J., Starr, J.M., 2013. Thyroid stimulating hormone, free thyroxine and cognitive ability in old age: the Lothian birth cohort study 1936. Psychoneuroendocrinology 38 (4) 597—601.

Castellano, C.-A., Laurin, D., Langlois, M.-F., Fortier, M., Tessier, D., Gaudreau, P., Ferland, G., Payette, H., Lorrain, D., Cunnane, S.C., 2013. Thyroid function and cognition in the euthyroid elderly: a case-control study embedded in Quebec longitudinal study—NuAge. Psychoneuroendocrinology 38 (9) 1772—1776.

Correia, N., Mullally, S., Cooke, G., Tun, T.K., Phelan, N., Feeney, J., Fitzgibbon, M., Boran, G., O'Mara, S., Gibney, J., 2009. Evidence for a specific defect in hippocampal memory in overt and subclinical hypothyroidism. J. Clin. Endocrinol. Metab. 94 (10) 3789—3797.

Fliers, E., Alkemade, A., Wiersinga, W.M., Swaab, D.F., 2006. Hypothalamic thyroid hormone feedback in health and disease. Prog. Brain Res. 153, 189—207.

Fox, M.D., Raichle, M.E., 2007. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat. Rev. Neurosci. 8, 700—711.

Gärtner, R., Gasnier, B.C.H., Dietrich, J.W., Krebs, B., Angstwurm, W.A., 2002. Selenium supplementation in patients with autoimmune thyroiditis decreases thyroid peroxidase antibodies concentrations. J. Clin. Endocrinol. Metab. 87, 1687—1691.

Grabe, H.J., Völzke, H., Lüdemann, J., Wolff, B., Schwahn, C., John, U., Meng, W., Freyberger, H.J., 2005. Mental and physical complaints in thyroid disorders in the general population. Acta Psychiatr. Scand. 112, 286—293.

Gronwall, D.M.A., 1977. Paced auditory serial-addition task: a measure of recovery from concussion. Perceptual Motor Skills 44, 367—373.

Hautzinger, M., Bailer, M., Worall, H., Keller, F., 1994. Beck—Depressions—Inventar (BDI). Hans Huber Verlag, Bern.

Hayasaka, S., Phan, K.L., Liberzon, I., Worsley, K.J., Nichols, T.E., 2004. Nonstationary cluster-size interference with random field and permutation methods. NeuroImage 22, 676—687.

Hogervorst, E., Huppert, F., Matthews, F.E., Brayne, C., 2008. Thyroid function and cognitive decline in the MRC cognitive function and ageing study. Psychoneuroendocrinology 33, 1013—1022.

de Jong, F.J., den Heijer, T., Visser, T.J., de Rijke, Y.B., Drexhage, H.A., Hofman, A., Bretteler, M.M.B., 2006. Thyroid hormones, dementia, and atrophy of the medial temporal lobe. J. Clin. Endocrinol. Metab. 91 (7) 2569—2573.

Kaya, M., Cermik, T.F., Bedel, D., Kutucu, Y., Tuglu, C., Yigitbasi, Ö.N., 2007. Assessment of alterations in regional cerebral blood flow in patients with hypothyroidism due to Hashimoto's thyroiditis. J. Endocrinol. Invest. 30, 491—496.

Kong, W.M., Sheikh, M.H., Lumb, P.J., Freedman, D.B., Crook, M., Doré, C.J., Finer, N., 2002. A 6-month randomized trial of thyroxine treatment in women with mild subclinical hypothyroidism. Am. J. Med. 112, 348—354.

Kramer, C.K., von Mühlen, D., Kritz-Silversetin, D., Barrett-Connor, E., 2009. Treated hypothyroidism, cognitive function, and depressed mood in old age: the Rancho Bernardo Study. Eur. J. Endocrinol. 161, 917—921.

Kratzsch, J., Schubert, G., Pulzer, F., Pfaeffle, F., Pfaeffle, R., Koerner, A., Dietz, A., Rauh, M., Kiess, W., Thiery, J., 2008. Reference intervals for TSH and thyroid hormones are mainly affected by age, body mass index and number of blood leucocytes, but hardly by gender and thyroid autoantibodies during the first decade of life. Clin. Biochem. 41, 1091—1098.

Krausz, Y., Freedman, N., Lester, H., Newman, J.P., Barkai, G., Bocher, M., Chisin, R., Bonne, O., 2004. Regional cerebral blood flow in patients with mild hypothyroidism. J. Nucl. Med. 45 (10) 1712—1715.

Ladenson, P.W., 2002. Psychological wellbeing in patients. Clin. Endocrinol. 57, 575—576.

Leyhe, T., Ethofer, T., Bretscher, J., Künle, A., Säuberlich, A.-L., Klein, R., Gallwitz, B., Häring, H.-U., Fallgatter, A., Klingberg, S., Sauer, R., Müssig, K., 2013. Low performance in attention testing is associated with reduced grey matter density of the left inferior frontal gyrus in euthyroid patients with Hashimoto's thyroiditis. Brain Behav. Immun. 27 (1) 33—37.

Leyhe, T., Müssig, K., Weinert, C., Laske, C., Häring, H.-U., Saur, R., Klingberg, S., Gallwitz, B., 2008. Increased occurrence of weaknesses in attention testing in patients with Hashimoto's thyroiditis compared to patients with other thyroid illnesses. Psychoneuroendocrinology 33, 1432—1436.

McMillan, C., Bradley, C., Razvi, S., Weaver, J., 2008. Evaluation of new measures of the impact of hypothyroidism on quality of life and symptoms: the ThyDQoL and ThySRQ. Value Health 11 (2) 285—294.

Miller, K.J., Parsons, T.D., Whybrow, P.C., van Herle, K., Rasgon, N., van Herle, A., Martinez, D., Silverman, D.H., Bauer, M., 2006. Memory improvement with treatment of hypothyroidism. Int. J. Neurosci. 116, 895—906.

Nagamachi, S., Jinnouchi, S., Nishii, R., Ishida, Y., Fujita, S., Futami, S., Kodama, T., Tamura, S., Kawai, K., 2004. Cerebral blood flow

abnormalities induced by transient hypothyroidism after thyroidectomy. Ann. Nucl. Med. 18 (6) 469–477.
Niemann, H., Sturm, W., Thöne-Otto, A., Willmes, K., 2008. California Verbal Learning Test (CVLT), Deutsche Adaptation. Pearson Assessment & Information GmbH, Frankfurt am Main.
Ott, J., Promberger, R., Kober, F., Neuhold, N., Tea, M., Huber, J.C., Hermann, M., 2011. Hashimoto's thyroiditis affects symptom load and quality of life unrelated to hypothyroidism: a prospective case-control study in women undergoing thyroidectomy for benign goiter. Thyroid 21 (2) 161–167.
Panicker, V., Saravanan, P., Vaidya, B., Evans, J., Hattersley, A.T., Frayling, T.M., Dayan, C., 2009. Common variation in the DIO2 gene predicts baseline psychological well-being and response to combination of thyroxine plus triiodothyronine therapy in hypothyroid patients. J. Clin. Endocrinol. Metab. 94, 1623–1629.
Piga, M., Serra, A., Deiana, L., Loi, G.L., Satta, L., Di Liberto, M., Mariotti, S., 2004. Brain perfusion abnormalities in patients with euthyroid autoimmune thyroiditis. Eur. J. Nucl. Med. Mol. Imag. 31 (12) 1639–1644.
Quidé, Y., Witteveen, A.B., El-Hage, W., Veltman, D.J., Olff, M., 2012. Differences between effects of psychological versus pharmacological treatments on functional and morphological brain alterations in anxiety disorders and major depressive disorder: a systematic review. Neurosci. Biobehav. Rev. 36, 626–644.
Quinque, E.M., Villringer, A., Kratzsch, J., Karger, S., 2013. Patient-reported outcomes in adequately treated hypothyroidism—insights from the German versions of ThyDQoL, ThySRQ and ThyTSQ. Health Qual. Life Outcomes 11, 68.
Reitan, R., Wolfson, D., 1993. The Halstead–Reitan Neuropsychology Test Battery: Theory and Clinical Interpretation, second ed. Neuropsychology Press, Tucson, AZ.
Samuels, M.H., Schuff, K.G., Carlson, N.E., Carello, P., Janowsky, J.S., 2007. Health status, psychological symptoms, mood, and cognition in l-thyroxine-treated hypothyroid subjects. Thyroid 17 (3) 249–258.
Saravanan, P., Chau, W.-F., Roberts, N., Vedhara, K., Greenwood, R., Dayan, C.M., 2002. Psychological well-being in patients on 'adequate' doses of l-thyroxine: results of a large, controlled community-bases questionnaire study. Clin. Endocrinol. 57, 577–585.
Schmidt, K.-H., Metzler, P., 1992. Wortschatztest (WST). Beltz Test GmbH, Weinheim.
Schraml, F.V., Beason-Held, L.L., 2010. Technetium-99m ethyl cysteinate dimer (ECD) cerebral accumulation and symptom and sign severity during hypothyroidism. Neuro. Endocrinol. Lett. 31 (1) 161–167.
Singh, S., Modi, S., Bagga, D., Kaur, P., Shankar, L.R., Khushu, S., 2013. Voxel-based morphometry analysis in hypothyroidism using diffeomorphic anatomic registration via an exponentiated lie algebra algorithm approach. J. Neuroendocrinol. 25, 229–234.
Sperling, R.A., Chua, E., Cocchiarella, A., Rand-Giovannetti, E., Poldrack, R., Schacter, D.L., Albert, M., 2003. Putting names to faces: successful encoding of associative memories activates the anterior hippocampal formation. NeuroImage 20, 1400–1410.
Van Dijk, K.R.A., Hedden, T., Venkataraman, A., Evans, K.C., Lazar, S.W., Buckner, R.L., 2010. Intrinsic functional connectivity as a tool for human connectomics: theory, properties, and optimization. J Neurophysiol. 103, 297–321.
Walsh, J.P., Ward, L.C., Burke, V., Bhagat, C.I., Shiels, L., Henley, D., Gillett, M.J., Gilbert, R., Tanner, M., Stuckey, B.G.A., 2006. Small changes in thyroxine dosage do not produce measurable changes in hypothyroid symptoms, well-being, or quality of life: results of a double-blind, randomized clinical trial. J. Endocrinol. Metab. 91, 2624–2630.
Wang, L., Hermens, D.F., Hickie, I.B., Lagopoulos, J., 2012. A systematic review of resting-state functional-MRI studies in major depression. J. Affective Disord. 142, 6–12.
Wechsler, D., 1987. Wechsler Memory Scale—Revised. Psychological Corporation, New York, NY.
Wekking, E.M., Appelhof, B.C., Fliers, E., Schene, A.H., Huyser, J., Tijssen, J.G.P., Wiersinga, W.M., 2005. Cognitive functioning and well-being in euthyroid patients on thyroxine replacement therapy for primary hypothyroidism. Eur. J. Endocrinol. 153, 747–753.
Wheeler, S.M., McAndrews, M.P., Sheard, E.D., Rovet, J., 2011. Visuospatial associative memory and hippocampal functioning in congenital hypothyroidism. J. Int. Neuropsychol. Soc. 18, 1–8.
Witthaus, E., Stewart, J., Bradley, C., 2001. Treatment satisfaction and psychological well-being with insulin glargine compared with NPH in patients with Type 1 diabetes. Diabetic Med. 18 (8) 619–625.
Zettinig, G., Asenbaum, S., Fueger, B.J., Hofmann, A., Diemling, M., Mittlboeck, M., Dudczak, R., 2003. Increased prevalence of subclinical brain perfusion abnormalities in patients with autoimmune thyroiditis: evidence of Hashimoto's encephalitis. Clin. Endocrinol. 59, 637–643.
Zhao, L., O'Neill, K., Diaz Brinton, R., 2005. Selective estrogen receptor modulators (SERMs) for the brain: current status and remaining challenges for developing NeuroSERMs. Brain Res. Rev. 49, 472–493.
Zhu, D.-F., Wang, Z.-X., Zhang, D.-R., Pan, Z.-L., He, S., Hu, X.-P., Chen, X.-C., Zhou, J.-N., 2006. fMRI revealed neural substrate for reversible working memory dysfunction in subclinical hypothyroidism. Brain 129, 2923–2930.
Zimmermann, P., Fimm, B., 2007. TAP Version 2.1: Testbatterie zur Aufmerksamkeitsprüfung. Psytest, Herzogenrath.

3 Treatment initiation effects

In addition to the long-term treated patient group described in publication 2, we have also studied a newly-diagnosed patient group to investigate treatment initiation effects. Acquisition and analysis procedures were almost identical to those reported in publication 2. Therefore, I will here only report specifics of this study arm.

Sample

Eleven patients newly diagnosed with hypothyroidism due to autoimmune thyroiditis were recruited via local endocrinologists, screening of blood donors and among participants of an ongoing large population-based study (LIFE study). One patient had to be excluded due to a benign brain anomaly, another because hypothyroidism could not be confirmed at study entry. Nine healthy control subjects (as described in detail in publication 2) were selected to match the patients in age, sex and intelligence.

Data analysis

Data of the first session was compared between groups to establish alterations in untreated hypothyroidism by means of t-tests for independent samples. Treatment initiation effects were assessed by paired t-tests to measure changes in the patient group between the sessions. Moreover, the interaction between session and group was calculated from analyses of covariance in order to investigate whether changes over the sessions were specific to the patient group. Lastly, the second session was compared between groups in order to find out whether patients reverted to a control

group level with treatment, again by means of t-tests for independent samples. All analyses were controlled for age and sex. Pearson correlations were used to correlate changes in mood and biochemical thyroid status with change in brain structure and function.

Results

In the final sample, nine newly diagnosed patients and nine healthy control subjects were included (see table 1 for an overview of sample characteristics). All participants were free of lifetime psychiatric or neurological illnesses, one patient received constant hypertensive medication. Data for one patient each were missing for the memory fMRI task and the resting-state fMRI due to technical problems and panicking, respectively. A corresponding control subject was excluded for the respective analyses to maintain matching of the groups.

Table 1: Sample characteristics and results of group comparisons at both sessions (pre/post treatment), changes between the sessions in the patient group, and the interaction between session and group.

	Patient group n=9		Control group n=9		*Group comparison	**Change	***Interaction
Age (years)	42±5		38±8		n.s.		
Sex	1 male		1 male		n.a.		
Intelligence (IQ)	104±10		107±8		n.s.		
	pre	**post**	**pre**	**post**	**pre**	**post**	**patients**	
TSH (mU/l)	6.1±1	3.4±1	2.9±1	2.5±1	<.01	=.08	<.01	<.01
fT3 (pmol/l)	4.8±1	4.6±1	4.5±1	4.4±0.4	n.s.	n.s.	n.s.	n.s.
fT4 (pmol/l)	12.6±2	15.7±2	14.9±1	14.5±1	<.05	n.s.	<.01	<.01
TPO-ab (U/ml)	285±184	287±184	11±2	9±3	<.01	<.01	n.s.	n.s.

*p-values of t-tests between the groups at both sessions, **p-values of a paired t-test within the patient group on changes between the two sessions *** p-values of the interaction between session and group calculated by analyses of covariance. Reference range was 0.4-4.0 for thyroid stimulating hormone (TSH), 3.1-6.79 for free triiodothyronine (fT3), 12.8-20.4 for free thyroxine (fT4), < 34 for thyroid peroxidase antibodies (TPO-ab), Kratzsch et al., 2008. Abbreviations: n.s. – not significant, n.a. – not applicable.

Levels of TSH and fT4 were significantly altered in the hypothyroid state and reverted to normal levels with treatment, although there was still a trend (p=0.08) for higher TSH values in the patient group. fT3 was within the normal range throughout and was not altered with treatment, whereas TPO-ab levels were constantly higher in the patient group (see Tab. 1).

Table 2: Mood parameters pre- and posttreatment initiation in hypothyroid patients and healthy controls and results of group comparisons, changes with treatment in the patient group and interaction between session and group.

	Patient group n=9		Control group n=9		*Group comparison		**Change	***Inter-action
	pre	post	pre	post	pre	post	patients	
Beck depression inventory	6.0±5	3.0±2	2.8±3	2.6±2	=.10	n.s.	=.08	n.s.
Hamilton depression scale	6.4±4	2.6±2	3.8±3	2.6±2	n.s.	n.s.	<.01	=.09
WBQ-12	23±5	27±4	29±3	30±4	<.05	n.s.	<.05	n.s.
SCL-90	1.3±0.2	1.1±0.1	1.2±0.2	1.1±0.1	=.09	n.s.	<.05	=.06
Short Form 36 – physical health	55±6	53±10	55±4	55±3	n.s.	n.s.	n.s.	n.s.
Short Form36 – mental health	45±8	52±5	52±5	51±5	<.05	n.s.	<.05	<.05
ThySRQ	6.9±3	3.8±2	2.8±2	3.2±2	<.01	n.s.	<.01	<.05
ThyDQoL	1.2±0.7	1.8±0.7	1.6±0.7	1.6±0.6	n.s.	n.s.	<.05	<.05

*p-values of t-tests between the groups at both sessions, **p-values of a paired t-test within the patient group over the two sessions *** p-values of the interaction between session and group calculated by analyses of covariance. Abbreviations: n.s. – not significant, WBQ – Well-Being Questionnaire, SCL – Symptom Check List, ThySRQ –Underactive Thyroid Symptom Rating Questionnaire, ThyDQoL – Thyroid-Dependent Quality of Life Questionnaire.

Patients showed reduced well-being (WBQ-12, Witthaus et al., 2001), perceived mental health (Short Form 36-mental, Bullinger and Kirchberger, 1998) and more hypothyroidism-related symptoms (ThySRQ, McMillan et al., 2008) compared to the control group and a trend for higher depressivity (Beck depression inventory, Haut-

zinger et al., 1994) and more psychological strain (SCL-90, Franke, 2002) in the hypothyroid state. After treatment initiation differences to the control group were no longer detected. All but perceived physical health improved in the patient group and the interaction between session and group was significant for a subset of measures (see Tab. 2).

There were no significant group differences in performance in any of the cognitive tasks at any session and no significant interaction, but an expected learning effect over time for a subset of tasks in both groups (data not shown). We did not find any differences in grey matter density even before treatment onset, unspecific changes over time in both groups, but no interaction between session and group (data not shown). We found no change with treatment in resting-state functional networks in the patient group nor was there a significant interaction. Two connectivity differences at session one turned out to be caused by unspecific changes in the control group (data not shown). In the fMRI memory task, patients outperformed the healthy control group behaviourally at session two (percent correct patient group: 73±8, control group: 62±11; $p<0.05$), the same descriptive trend was there at session one (percent correct patient group: 70±9, control group: 64±8, $p>0.1$). As interpretability of the functional data would thus be very limited, we did not analyse the task-based fMRI data further. Planned correlation analyses of the change in brain structure and function with changes in thyroid hormone levels and mood showed no coherent relationship (data not shown).

Discussion

We found subclinically reduced mood in mildly hypothyroid patients that recovered with treatment. In contrast, we found no evidence for cognitive deficits, grey matter

alterations or functional connectivity changes and no systematic associations with thyroid hormone levels or mood. The observed mood normalisation with treatment possibly mirrors a placebo effect as a recent review (Dayan and Panicker, 2013) has shown that the effect is only observed in studies employing healthy control groups as used here, but not when compared to placebo control groups.

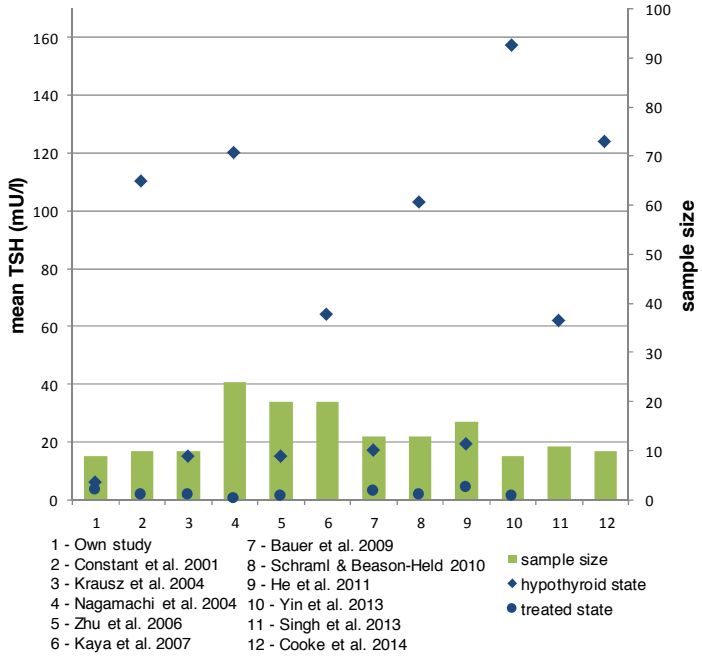

Figure 9: Comprehensive summary of brain imaging studies on hypothyroidism, depicting sample sizes (green bars) and TSH levels (blue symbols) in the hypothyroid and treated state (if available).

Although sample size was small (n=9), it is comparable to other clinical brain imaging studies in the field that were able to show differences in untreated hypothyroidism and partly changes with treatment (see Fig. 9, green bars). However, our subjects showed milder hypothyroidism at study entry and accordingly lower changes in thyroid hormone levels with treatment (see Fig. 9, blue symbols). From an experi-menttal perspective, our patient group is ill-suited to show proof of principle of

changes due to hormonal action, as the effect size of the independent variable is small. From a clinical perspective however, this group is especially interesting as it is still debated whether to treat or not to treat mild subclinical hypothyroidism (Owen and Lazarus, 2003; Vanderpump, 2003).

In addition to altered thyroid hormone levels, the patient group showed significantly heightened TPO-ab activity throughout the study. However, this did not lead to significant group differences. This does not support the idea of an independent influence of thyroid autoimmunity on cognition, mood and brain (Gärtner et al., 2002; Leyhe et al., 2008), but given the limited power of the study, may as well be a false negative finding.

In summary, in this study on treatment initiation in mild autoimmune hypothyroidism we cannot rule out the possibility of a false negative finding in cognition, brain structure and function nor a placebo effect concerning mood normalisation and hence cannot draw any definite conclusions. In order to avoid publication bias in the field commonly suffering from low power due to recruitment difficulties, we have made our dataset publically available for sharing to improve meta-analyses (Poldrack and Gorgolewski, 2014; http://neurovault.org/collections/169/).

IV Discussion and Outlook

The experimental findings have already been discussed in the respective sections. Here, I will therefore concentrate on more general issues that have arisen during the course of the research project.

Sample size in both study arms of the MRI study was small (n=18 treated patients, n=9 newly diagnosed patients), but comparable to other MRI studies in the field. Low power is thus an endemic problem of the research area, mainly performed in developed countries where hypothyroidism is usually detected at an early stage, so that severe cases are rare. Additional constraints due to either requirements of the MR technique (no ferromagnetic implants, no tattoos) or experimental considerations (age limit, exclusion of comorbidities) further limit the number of available subjects. Therefore, options to increase subject numbers should be considered for future studies in order to increase the robustness of findings. Firstly, data from large population-based studies may be used (see also discussion below and outlook). Secondly, multicentre studies may be initiated. Thirdly, data should be made available at adequate levels of detail for later meta-analyses, including null findings to avoid bias. The NeuroVault project allows the publishing of unthresholded statistical maps of brain imaging data to share results at a whole-brain level rather than just reporting peak activations (Poldrack and Gorgolewski, 2014). We have uploaded results of both MRI study arms there (http://neurovault.org).

Interest in thyroid function has been high in large population-based samples that have recently emerged thanks to computational advances in handling such large datasets. Many were published concurrently with or after the current study, resulting in substantial new evidence concerning hypothyroidism and both mood (7 studies

with 1000 to 33.000 participants) and cognition (11 studies with 81 to 2000 participants) that are relevant to our own findings and shall thus be discussed below. The studies consistently report impairment of mood independent of biochemical thyroid hormone levels in long-term treated patients (Jørgensen et al., 2014; Panicker et al., 2009b; van de Ven et al., 2012a). In contrast, people with unknown incidental hypothyroidism or high normal TSH values did not show reduced mood (de Jongh et al., 2011; Jørgensen et al., 2014; Klaver et al., 2013; Kvetny et al., 2014; Medici et al., 2014; Panicker et al., 2009b; van de Ven et al., 2012a, 2012b). The authors consistently conclude that unspecific factors such as comorbidities leading to selection bias in seeking health care or the awareness of having a chronic disease cause the mood disturbance observed in clinical but not in population-based samples. However, another possible cause was suggested by Panicker et al. (2009a). In treated patients brain hypothyroidism may persist despite serum hormone levels in the reference range, because of genetic differences in deiodinase enzymes needed to transform the artificial fT4, standard treatment for hypothyroidism, into the neurally active fT3 in the brain. We were, however, not able to find brain alterations in treated patients that would support this hypothesis and several attempts to administer T4/T3 combination therapy to eliminate possible brain hypothyroidism have been unsuccessful (see Grozinsky-Glasberg et al., 2006 for a review). Effects may be limited to carriers of specific polymorphisms though, hidden when investigated in unselected samples (Panicker et al., 2009a; Wiersinga et al., 2009). This line of research therefore warrants further study in larger samples (see outlook).

Concerning cognition, two comparably small studies (n<500) have found an association between hypothyroidism and cognitive impairment (Johnson et al., 2011; Resta et al., 2012), but most have not (Booth et al., 2012; Castellano et al., 2013;

Formiga et al., 2014; Forti et al., 2012; de Jongh et al., 2011; Moon et al., 2014; Parsaik et al., 2014). Overall, effects were small or absent independent of treatment status in line with our own findings in treated as well as newly diagnosed patients. Although our own findings may be false negative due to the small sample size, the overwhelming evidence from well-powered studies suggests that mild hypothyroidism does indeed not cause clinically significant cognitive impairment as suggested by early clinical studies (Baldini et al., 1997; Burmeister et al., 2001).

Lastly, it should be noted that other factors than cognitive and affective symptoms as studied here are important for clinical treatment decisions. Most importantly, over-treatment resulting in hyperthyroidism has been associated with increased cardio-vascular mortality and reduced bone mineral density. In contrast, hypothyroidism has been associated with an increased risk for atherosclerosis and diabetes. Comprehen-sive assessment of comorbidities and side effects of treatment thus need thorough monitoring (Duntas et al., 2012; Owen and Lazarus, 2003; Vanderpump, 2003).

Outlook

Only one of the population-based studies looking at thyroid function has included brain imaging data (Rotterdam Scan Study, de Jong et al., 2006, 2007). The authors however report only on the volumes of two brain areas of interest, hippocampus and amygdala. Given that additional brain areas may be affected by hypothyroidism (e.g. Bauer et al., 2009) and not only structural, but also functional alterations may occur (e.g. Zhu et al., 2006), large population-based studies including whole-brain structural and functional MRI are needed to validate hypotheses generated by small clinical samples with sufficient statistical power. Fortunately, such a dataset has recently been acquired (LIFE study, Leipzig) and we have already obtained permis-

sion to analyse the data. Ideally, we will additionally obtain genetic data on polymorphisms in the genes coding for deiodinases. Should polymorphisms indeed influence the availability of thyroid hormones at the brain level (Panicker et al., 2009a; Dayan and Panicker, 2013), this should result in differences in brain structure and function as measurable with MRI in thyroid healthy people, but even more so in levothyroxine-treated patients and may possibly explain residual mood alterations.

In addition, we found autoimmunity and disease duration to be associated with neural structure and function in long-term adequately treated patients. An obvious future line of research would therefore be to follow up on studies using selenium supplementation to reduce TPO-ab in autoimmune thyroiditis and investigate possible neural underpinnings of the observed mood improvement (Gärtner et al., 2002). In order to further study effects of disease duration, a longitudinal study over a longer time scale in adequately treated patients would be required.

Finally, I would like to mention an anecdotal observation made during the study with implications for current recommendations for levothyroxine administration. Many participating patients reported that the recommended intake time half an hour before breakfast is the most bothering consequence of the disease, reducing quality of life. However, a recent randomised double-blind crossover study (Bolk et al., 2010) showed that levothyroxine uptake was even slightly better when taken before bedtime, possibly due to eating habits such as coffee consumption at breakfast. Interestingly, patients were allowed to choose their preferred intake time after the study ended. After one year, the majority preferred intake at bedtime. The study was performed in the Netherlands and described eating habits are similar in Germany. The promising pioneer study should thus be replicated in an independent sample and recommendations for levothyroxine intake time may then require reconsideration.

V Summary

Zusammenfassung der Arbeit

Dissertation zur Erlangung des akademischen Grades Dr. rer. med.

Brain, mood and cognition in hypothyroidism

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März 2015

1 English Summary

Hypothyroidism during foetal brain development leads to severe and irreversible mental retardation. Large-scale public health efforts to reduce iodine deficiency have, however, largely eliminated the condition (Führer et al., 2014; Pearce et al., 2013). Adult-onset hypothyroidism has received comparably much less attention, despite consistent reports about mood and cognitive impairment (Canaris et al., 1997; Joffe et al., 2012). Prevalence is about 5%, with higher rates in women than men (Roberts and Ladenson, 2004). Residual symptoms have been reported in patients with biochemically adequate treatment (Samuels et al., 2007; Saravanan et al., 2002). Reasons discussed for residual symptoms include firstly independent effects of autoimmune processes on the brain, the most common cause of adult-onset hypothyroidism (Grabe et al., 2005), secondly brain hypothyroidism in spite of normal serum hormone levels (Panicker et al., 2009a), thirdly comorbidities leading to a

higher chance of having thyroid hormone values tested (Kong et al., 2002) and fourthly reactive mental processes to the awareness of having a chronic disease (Ladenson, 2002). Disentangling these possible causes has important implications for treatment strategies. The present study wants to contribute to the discussion by studying the target organ of interest, the brain, and individual levels of autoimmunity. Studies of the brain in hypothyroid rodents have shown impaired synaptic plasticity in the hippocampus associated with impaired spatial learning (Alzoubi et al., 2009) and neurophysiological changes in the amygdala and hippocampus related to increased anxiety and depression (Montero-Pedrazuela et al., 2006, 2011). However, little is known about neural correlates of hypothyroidism-related symptoms in humans. First studies using PET and SPECT have shown changes in brain glucose metabolism and cerebral blood flow in hypothyroidism, but as of yet studies are inconsistent concerning reversibility and localisation (Bauer et al., 2009; Constant et al., 2001; Krausz et al., 2004). Magnetic resonance imaging (MRI) is a powerful non-invasive tool to investigate the effects of hormone action on structure and function of the brain in living patients (Brabant et al., 2011; Pilhatsch et al., 2011) and has been used to study neural correlates of hypothyroidism here. We were interested both in initial treatment effects of levothyroxine to reestablish euthyroidism as well as in long-term treatment effects to investigate possible causes for residual symptoms. In order to study independent effects of the autoimmune process, we have included patients with hypothyroidism of autoimmune genesis and have measured thyroid peroxidase antibodies (TPO-ab) in addition to thyroid hormone values.

As a methodological prerequisite for the main MRI study, we firstly translated a set of questionnaires on symptoms, quality of life and treatment satisfaction in hypothyroidism (McMillan et al., 2006; 2008) and validated them in an independent

sample (n=101). Reliability and structural validity was satisfactory for all three questionnaires (Quinque et al., 2013, publication 1).

In the first arm of our MRI study, we investigated 18 long-term adequately treated patients with autoimmune hypothyroidism and 18 matched healthy control subjects. We applied structural and functional MRI as well as neuropsychological testing to assess mood and cognition. For the analysis of brain structure, we used voxel-based morphometry (Ashburner and Friston, 2000), that allows voxel-wise comparison of grey matter density values between groups. In addition we performed an associative memory task using functional MRI (fMRI) in order to investigate hippocampus-dependent brain activity. Finally, we applied seed-based resting-state fMRI (Fox and Raichle, 2007) to study neural connectivity of brain regions that are known to be altered in hypothyroidism (Bauer et al., 2009).

Results showed that adequately treated patients report more symptoms, higher depressivity and reduced well-being as compared to healthy control subjects (Quinque et al., 2013, publication 1). However, these mood alterations were not related to brain structure and function in depression-specific brain regions. Furthermore, we did not find differences between patient and control group in cognitive performance, brain structure, functional connectivity or brain activity during associative memory encoding. We did however find higher TPO-ab levels to be associated with higher grey matter density in the right amygdala and increased connectivity between subcallosal cortex and left parahippocampal gyrus, all part of a depression and fear network (Quidé et al., 2012). Moreover, treatment duration was related to structural and functional changes in areas associated with depression and untreated hypothyroidism. Therefore, we identified autoimmunity and treatment duration as

possible factors to explain neural sources of residual symptoms in long-term treated hypothyroidism (Quinque et al., 2014, publication 2).

In the second study arm we investigated 9 newly diagnosed patients before and 3 months after treatment initiation and 9 healthy matched control subjects. Patients showed mild hypothyroidism that reverted to normal levels with treatment. The groups did not differ significantly in cognitive performance or brain structure and function, even before treatment. However, patients reported reduced mood that reverted to control group levels with treatment. This may be due to a placebo effect described in the literature (Dayan and Panicker, 2013). Compared to studies that showed brain alterations in the untreated state (Bauer et al., 2009; Singh et al., 2013), the current sample showed milder hypothyroidism (TSH (mU/l) 6 vs. 17 and 124, respectively), but was of a similar size (n=9 vs. 13 and 10, respectively). Therefore, we can currently neither rule out a false negative finding nor a true lack of alterations in mild hypothyroidism as suggested by recent large population-based studies (Jørgensen et al., 2014; Kvetny et al., 2014; Moon et al., 2014). We have made our dataset publically available (http://neurovault.org) in order to contribute data to the field commonly relying on small patient samples.

Future studies on the neural correlates of hypothyroidism should use larger samples from population-based studies or by pooling data across studies to avoid power problems endemic to the resource-intensive MRI method. Effects of autoimmunity and disease duration should be followed up. In mildly hypothyroid or long-term adequately treated patients presenting with residual symptoms, alternative causes such as reactive mental processes to the awareness of having a chronic disease should be considered.

2 German Summary

Eine Hypothyreose während der fötalen Hirnentwicklung, meist bedingt durch starken Iodmangel, führt zu schwerer und irreversibler geistiger Behinderung, bekannt als Kretinismus. Kretinismus ist dank weltweiter Programme zur Iodierung von Speisesalz inzwischen sehr selten (Führer et al., 2014; Pearce et al., 2013). Der Hypothyreose mit Beginn im Erwachsenenalter wurde vergleichsweise weniger Aufmerksamkeit zuteil, obwohl auch für diese affektive und kognitive Beeinträchtigungen berichtet werden (Canaris et al., 1997; Joffe et al., 2012). Die Prävalenz für klinische oder subklinische Hypothyreose beträgt etwa 5%. Frauen sind etwa doppelt so häufig betroffen wie Männer (Roberts and Ladenson, 2004). Symptome wurden auch in adäquat hormonsubstituierten Patienten berichtet (Samuels et al., 2007; Saravanan et al., 2002). Mögliche Gründe hierfür sind erstens unabhängige Effekte von Autoimmunprozessen auf das Gehirn, der häufigsten Ursache für eine Hypothyreose im Erwachsenenalter (Grabe et al., 2005), zweitens Schilddrüsenhormonmangel im Gehirn trotz normaler peripherer Schilddrüsenhormonwerte (Panicker et al., 2009a), drittens Komorbiditäten, die zu einer höheren Wahrscheinlichkeit führen, dass als Nebenbefund eine Hypothyreose diagnostiziert wird (Kong et al., 2002), oder viertens reaktive psychische Prozesse auf die Diagnose einer chronischen Erkrankung (Ladenson, 2002). Die Analyse dieser möglichen Ursachen hat wichtige Implikationen für Behandlungsstrategien. Die vorliegende Studie möchte einen Beitrag zu dieser Diskussion leisten, indem das interessierende Zielorgan, das Gehirn, sowie die Stärke der Autoimmunaktivität untersucht werden. Studien an den Gehirnen hypothyreoter Nagetiere zeigten Beeinträchtigungen in der synaptischen Plastizität im Hippocampus, assoziiert mit Defiziten im räumlichen Lernen (Alzoubi et al., 2009), sowie neurophysiologische Veränderungen in Amygdala und Hippocam-

pus in Verbindung mit erhöhter Angst und Depression (Montero-Pedrazuela et al., 2006, 2011). Zu neuronalen Korrelaten der Hypothyreose bei Menschen ist weniger bekannt. Erste Studien mit PET und SPECT zeigten Veränderung im zerebralen Glukosestoffwechsel sowie im Blutfluss, waren jedoch inkonsistent in Bezug auf Reversibilität und Lokalisation der Veränderungen (Bauer et al., 2009; Constant et al., 2001; Krausz et al., 2004).

Im vorliegenden Projekt haben wir die nicht-invasive Magnetresonanztomographie (MRT) genutzt, um neuronale Korrelate der Hypothyreose beim Menschen zu untersuchen. Wir interessierten uns dabei für Korrelate der akuten Hypothyreose, für initiale Behandlungseffekte mit Levothyroxin zur Wiederherstellung der Euthyreose und für Effekte einer adäquaten Langzeitbehandlung, um Gründe für mögliche persistierende Symptome zu untersuchen. Um unabhängige Effekte des Autoimmunprozesses zu analysieren, untersuchten wir Patienten mit einer Hypothyreose autoimmuner Genese und erhoben Thyreoperoxidase- (TPO) Antikörper-Titer neben den Schilddrüsenhormonwerten.

Als methodische Vorarbeit für die MRT-Studie haben wir Fragebögen zu den Themen Symptome, Lebensqualität sowie Behandlungszufriedenheit bei Hypothyreose übersetzt (McMillan et al., 2006; 2008) und an einer unabhängigen Stichprobe (n=101) validiert. Reliabilität und Konstruktvalidität waren zufriedenstellend (Quinque et al., 2013, Publikation 1).

Im ersten Teil der MRT-Studie untersuchten wir 18 aktuell euthyreote Patienten mit autoimmuner Hypothyreose unter langjähriger Hormonsubstitution sowie 18 gesunde Kontrollprobanden. Erhoben wurden strukturelle und funktionelle MRT Daten, neuropsychologische Testverfahren zur Messung von kognitiver Performanz, sowie Fragebögen zur Erfassung von selbstberichteter Stimmung. Für die Analyse von

Hirnstrukturen haben wir die Methode der voxel-basierten Morphometrie verwendet (Ashburner and Friston, 2001). Die Methode erlaubt den voxelweisen Vergleich der Dichte der grauen Substanz. Zusätzlich haben wir mittels funktionellem MRT (fMRT) die Gehirnaktivität, besonders die im Hippocampus, während eines assoziativen Gedächtnistests untersucht. Zudem haben wir die sogenannte „seed-based resting state“ fMRT Methode genutzt (Fox and Raichle, 2007), um die neuronale Konnektivität der Hirnareale zu untersuchen, die veränderten Metabolismus bei Hypothyreose gezeigt haben (Bauer et al., 2009).

Im Vergleich zur Kontrollgruppe berichteten adäquat behandelte Patienten mehr Symptome, höhere Depressivität und reduziertes Wohlbefinden (Quinque et al., 2013, Publikation 1). Diese Stimmungsveränderungen waren jedoch nicht mit Hirnstruktur oder -funktion in depressionsspezifischen Arealen assoziiert. Die Patienten unterschieden sich nicht von der Kontrollgruppe in der kognitiven Performanz, Hirnstruktur, neuronalen Konnektivität oder Hirnaktivität während eines Gedächtnistests. Die Höhe der TPO-Antikörper-Titer war jedoch mit erhöhter Dichte der grauen Substanz in der rechten Amygdala sowie mit einer erhöhten neuronalen Konnektivität zwischen dem Cortex Subcallosus und dem linken parahippocampalen Gyrus korreliert. Alle genannten Regionen gehören einem Depressions- und Angstnetzwerk an (Quidé et al., 2012). Zudem war die Erkrankungsdauer mit strukturellen und funktionellen Veränderungen in Arealen korreliert, die mit Depression und unbehandelter Hypothyreose in Verbindung gebracht werden. Daher haben wir Autoimmunität und Erkrankungsdauer als mögliche Faktoren zur Erklärung neuronaler Ursachen für residuale Symptome bei chronischer behandelter Autoimmunthyreoiditis identifiziert (Quinque et al., 2014, Publikation 2).

Am zweiten Teil der MRT-Studie nahmen 9 neu diagnostizierte Patienten mit autoimmuner Hypothyreose vor und 3 Monate nach Beginn der Hormonsubstitution mit Levothyroxin teil, sowie 9 gesunde Kontrollprobanden. Die Patienten waren zu Studienbeginn leicht hypothyreot, bei der Wiederholungsmessung waren alle Serumhormonwerte im Normbereich. Die Gruppen unterschieden sich bei keinem Messzeitpunkt signifikant in der kognitiven Performanz, Hirnstruktur oder -funktion. Die Patienten berichteten jedoch reduzierte Stimmung, die sich mit der Medikation normalisierte. Dabei könnte es sich jedoch um einen in der Literatur beschriebenen Placebo-Effekt handeln (Dayan and Panicker, 2013). Im Vergleich zu hirnbildgebenden Studien, die signifikante Gruppenunterschiede im Zustand vor der Behandlung gezeigt haben (Bauer et al., 2009; Singh et al., 2013), wies unsere Patientengruppe eine mildere Form der Hypothyreose auf (TSH (mU/l) 6 versus 17 bzw. 124), die Stichprobengröße war jedoch vergleichbar (n=9 versus 13 bzw. 10). Wir können daher derzeit weder ein falsch negatives Ergebnis noch die Abwesenheit von neuronalen Veränderungen bei milder Hypothyreose ausschließen, wie es aktuelle populationsbasierte Studien nahelegen (Jørgensen et al., 2014; Kvetny et al., 2014; Moon et al., 2014). Wir haben die Daten öffentlich zugänglich gemacht (http://neurovault.org), um den Publikationsbias in dem Forschungsgebiet, das häufig mit kleinen Patientenkohorten arbeitet, zu vermindern.

Zukünftige Studien zu neuronalen Korrelaten der Hypothyrose sollten möglichst größere Stichproben aus populationsbasierten Studien oder gepoolte Daten aus mehreren Studien nutzen, um das endemische Problem geringer statistischer Power der ressourcenintensiven MRT-Methode zu verringern. Autoimmunität und Erkrankungsdauer sollten dabei weiter untersucht werden. Bei Patienten mit subklinischer Hypothyreose sowie bei adäquat eingestellten Patienten, die über

residuale Stimmungsveränderungen klagen, sollten aber auch andere mögliche Ursachen wie beispielsweise Komorbiditäten oder reaktive psychische Prozesse auf die Diagnose einer chronischen Erkrankung in der Behandlungsstrategie mit berücksichtigt werden.

VI References

Allolio B. & Schulte H.M. (Eds.). (1996). *Praktische Endokrinologie.* Urban & Schwarzenberg: München, Wien, Baltimore.

Alzoubi, K.H., Gerges, N.Z., Aleisa, A.M. & Alkadhi, K.A. (2009). Levothyroxin restores hypothyroidism-induced impairment of hippocampus-dependent learning and memory: behavioral, electrophysiological, and molecular studies. *Hippocampus, 19,* 66-78.

Ambrogini, P., Cuppini, R., Ferri, P., Mancini, C., Ciaroni, S., Voci, A., Gerdoni, E. & Gallo, G. (2005). Thyroid hormones affect neurogenesis in the dentate gyrus of adult rat. *Neuroendocrinology, 81,* 244-253.

Artis, A.S., Bitiktas, S., Taşkin, E., Dolu, N., Liman, N. & Suer, C. (2011). Experimental hypothyroidism delays field excitatory post-synaptic potentials and disrupts hippocampal long-term potentiation in the dentate gyrus of hippocampal formation and Y-maze performance in adult rats. *J Neuroendocrinol, 24,* 422-433.

Ashburner, J. & Friston, K.J. (2000). Voxel-based morphometry – the methods. *NeuroImage, 11;* 805-821.

Baldini, M., Vita, A., Mauri, M.C., Amodei, V., Carrisi, M., Bravin, S. & Cantalamesse, L. (1997). Psychopathological and cognitive features in subclinical hypothyroidism. *Prog Neuropsychopharmacol Biol Psychiatry, 21,* 925-935.

Bauer, M., Silverman, H.S., Schlagenhauf, F., London, E.D., Geist, C.L., van Herle, K., Rasgon, N., Martinez, D., Miller, K., van Herle, A., Berman, S.M., Phelps, M.E, & Whybrow, P.C. (2009). Brain glucose metabolism in hypothyroidism: a positron emission tomography study before and after thyroid hormone replacement therapy. *J Clin Endocrinol Metab, 94*, 2922-2929.

Baumann, U. (1976). Methodische Untersuchungen zur Hamilton-Depressionsskala. *Arch Psychiatr Nervenkr, 222,* 359-375.

Beydoun, M.A., Beydoun, H.A., Shroff, M. R., Kitner-Triolo, M.H., & Zonderman, A.B. (2012). Serum leptin, thyroxine and thyroid-stimulating hormone levels interact to affect cognitive function among US adults: evidence from a large representative survey. *Neurobiol Aging, 33(8),* 1730-1743 .

Bianchi, G.P., Zaccheroni, V., Solaroli, E., Vescini, F., Cerutti, R., Zoli, M. & Marchesini, G. (2004). Health-related quality of life in patients with thyroid disorders. *Qual Life Res, 13;* 45-54.

Biswal, B., Yetkin, F.Z., Haughton, V.M. & Hyde, J.S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Res Med, 34,* 537-541.

Bjergved, L., Jørgensen, T., Perrild, H., Carlé, A., Cerqueira, C., Krejbjerg, A., Laurberg, P., Ovesen, L, Bülow Pedersen, I., Banke Rasmussen, L. & Knudsen, N. (2012). Predictors of change in serum TSH after iodine fortification: an 11-year follow-up to the DanThyr study. *J Clin Endocrinol Metab, 97(11),* 4022-4029.

Boelart, K. & Franklyn, J.A. (2005). Thyroid hormone in health and disease. *J Endocrinol, 187,* 1-15.

Bolk, N., Visser, T.J., Nijman, J., Jongste, I.J., Tijssen, J.G.P. & Berghout, A. (2010). Effects of evening vs morning levothyroxine intake. *Arch Intern Med, 170(22),* 1996-2003.

Bono, G., Fancellu, R., Blandini, F., Santoro, G. & Mauri, M. (2004). Cognitive and affective status in mild hypothyroidism and interactions with l-thyroxine treatment. *Acta Neurol Scand,* 110; 59-66.

Booth, T., Deary, I.J. & Starr, J.M. (2012). Thyroid stimulating hormone, free thyroxine and cognitive ability in old age: the Lothian birth cohort study 1936. *Psychoneuroendocrinology, 38(4),* 597-601.

Brabant, G., Cain, J., Jackson, A. & Kreitschmann-Andermahr, I. (2011). Visualizing hormones actions in the brain. *Trends Endocrinol Metab, 22(5),* 153-163.

Broedel, O., Eravci, M., Fuxius, S., Smolarz, T., Jeitner, A., Grau, H., Stoltenburg-Didinger, G., Plueckhan, H., Meinhold, H. & Baumgartner, A. (2003). Effects of hyper- and hypothyroidism on thyroid hormone concentrations in regions of the rat brain. *Am J Physiol Endocrinol Metab, 285,* E470-E480.

Buckner, R.L. & Logan, J.M. (2001). Chapter 2, Functional Neuroimaging Methods: PET and fMRI. In R. Cabeza & A. Kingstone (Eds.). *Handbook of Functional Neuroimaging of Cognition.* Cambridge, MA: MIT Press.

Bullinger, M. & Kirchberger, I. (1998). *SF-36. Fragebogen zum Gesundheitszustand Handanweisung.* Göttingen: Hogrefe.

Burmeister, L.A., Ganguli, M., Dodge, H.H., Toczek, T., DeKosky, S.T. & Nebes, R.D. (2001). Hypothyroidism and cognition: preliminary evidence for a specific defect in memory. *Thyroid, 11 (12),* 1177-1185.

Buxton, R.B. (2009). *Introduction to functional magnetic resonance imaging* (2nd ed.). Cambridge: Cambridge University Press.

Canaris, G.J., Steiner, J.F. & Ridgway, E.C. (1997). Do traditional symptoms of hypothyroidism correlate with biochemical disease? *J Gen Intern Med, 12;* 544-550.

Castellano, C.A., Laurin, D., Langlois, M.F., Fortier, M., Tessier, D., Gaudreau, P., Ferland, G., Payette, H., Lorrain, D. & Cunnane, S.C. (2013). Thyroid function and cognition in the euthyroid elderly: a case-control study embedded in Quebec longitudinal study – NuAge. *Psychoneuroendocrinology, 38(9),*1772-1776.

Caturegli, P., De Remigis, A., Chuang, K., Dembele, M., Iwama, A. & Iwama, S. (2013). Hashimoto Thyroiditis: celebrating the centennial through the lens oft he John Hopkins Hospital surgical pathology records. *Thyroid, 23(2),* 142-150.

Chaalal, A., Poirier, R., Blum, D., Gillet, B., Le Blanc, P., Basquin, M., Buée, M., Laroche, S. & Enderlin, V. (2014). PTU-induced hypothyroidism in rats leads to several early neuropathological signs of Alzheimer's disease in the hippocampus and spatial memory impairments. *Hippocampus, 24,* 1381-1393.

Constant, E.L., de Volder, A.G., Ivanoiu, A., Bol, A., Labar, D., Seghers, A., Cosnard, G., Melin, J. & Daumerie, C. (2001). Cerebral blood flow and glucose metabolism in hypothyroidism: a positron emission tomography study. *J Clin Endocrino. Metab, 86,* 3864-3870.

Cooke, G.E., Mullally, S., Correia, N., O'Mara, S.M. & Gibney, J. (2014). Hippocampal volume is decreased in adults with hypothyroidism. *Thyroid, 24(3),* 433-440.

Correia, N., Mullally, S., Cooke, G., Tun, T. K., Phelan, N., Feeney, J., Fitzgibbon, M., Boran, G., O'Mara, S. & Gibney, J. (2009). Evidence for a specific defect in hippocampal memory in overt and subclinical hypothyroidism. *J Clin Endocrinol Metab, 94(10),* 3789-3797.

Cortés, C., Eugenin, E., Aliaga, E., Carreňo, L.J., Bueno, S.M., Gonzalez, P.A., Gayol, S., Naranjo, D., Noches, V., Marassi, M.P., Rosenthal, D., Jadue, C., Ibarra, P., Keitel, C., Wohllk, N., Court, F., Kalergis, A.M. & Riedel, C.A. (2012). Hypothyroidism in the adult rat causes incremental changes in brain-derived neurotrophic factor (BDNF), neuronal and astrocyte apoptosis, gliosis and deterioration of postsynaptic density. *Thyroid,* 22(9), 951-963.

Dayan, C.M. & Panicker, V. (2009). Novel insights into thyroid hormones from the study of common genetic variation. *Nat Rev Endocrinol, 5,* 211-218.

Dayan, C.M. & Panicker, V. (2013). Hypothyroidism and depression. *Eur Thyroid J, 2,* 168-179.

Delange, F., Bürgi, H. & Moinier, B. (2004). Chapter 8, Iodine deficiency disorders (IDD) in western and central Europe. In B.S. Hetzel, F. Delange, J.T. Dunn, J. Ling, V. Mannar & C. Pandav (Eds.). *Towards the global elimination of brain damage due to iodine deficiency.* Delhi: Oxford University Press.

Desouza, L.A., Ladiwala, U., Daniel, S.M., Agashe, S., Vaidya, R.A. & Vaidya, V.A. (2005). Thyroid hormone regulates hippocampal neurogenesis in the adult rat brain. *Mol Cell Neurosci, 29,* 414-426.

Duntas, L.H., Amino, N., Hay, I., McDermott, M., Peeters, R., Vaismann, M., Ward, L., Williams, G., San Luis Jr., T.O.L. & Yen, P. (2012). Thyroid disorders, non-communicable diseases that gravely impact public health: a commentary and statement by the advisory board of the world thyroid federation. *Thyroid, 22(6),* 566-567.

Ehlert, U. & von Känel, R. (Hrsg.) (2011). *Psychoendokrinologie und Psychoimmunologie.* Berlin: Springer.

Engum, A., Bjøro, T., Mykletun, A. & Dahl, A.A. (2002). An association between depression, anxiety and thyroid function - a clinical fact or an artefact? *Acta Psychiatr Scand, 106,* 27-34.

Eurich, D.T., Johnson, J.E., Reid, K.J. & Spertus, J.A. (2006). Assessing responsiveness of generic and specific health related quality of life measures in heart failure. *Health Qual Life Outcomes, 4 (89).*

Fernández-Lamo, I., Montero-Pedrazuela, A., Delgado-García, J.M. & Guadaño-Ferraz, A. (2009). Effects of thyroid hormone replacement on associative learning and hippocampal synaptic plasticity in adult hypothyroid rats. *Eur J Neurosci, 30,* 679-692.

Formiga, F., Ferrer, A., Padros, G., Contra, A., Corbella, X. & Pujol, R. (2014). Thyroid status and functional and cognitive status at baseline and survival after 3 years of follow-up: the OCTABAIX study. *Eur J Endocrinol, 170,* 69-75.

Forti, P., Olivelli, V., Rietti, E., Maltoni, B., Pirazzoli, G., Gatti, R., Gioia, M.G. & Ravaglia, G. (2012). Serum thyroid-stimulating hormone as a predictor of cognitive impairment in an elderly cohort. *Gerontology, 58,* 41-49.

Fox, M.D. & Raichle, M.E. (2007). Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci, 8,* 700-711.

Franke, G.H. (2002). *SCL-90-R Symptomcheckliste von L.R. Derogatis – Deutsche Version, 2.Aufl.* Göttingen: Beltz Test GmbH.

Friston, K., Ashburner, J.T., Kiebel, S.J., Nichols, T.E. & Penny, W.D. (Eds.). (2007). *Statistical Parametric Mapping.* London: Academic Press.

Friston, K.J., Jezzard, P. & Turner, R. (1994). Analysis of functional MRI time-series. *Hum Brain Mapp, 1,* 153-171.

Fu, A. L., Zhou, C.Y. & Chen, X. (2010). Thyroid hormone prevents cognitive deficit in mouse model of Alzheimer's disease. *Neuropharmacology, 58,* 722-729.

Führer, D., Mann, K., Feldkamp, J., Krude, H., Spitzweg, C., Kratzsch, J. & Schott, M. (2014). Schilddrüsenfunktionsstörungen in der Schwangerschaft. *Dtsch Med Wochenschr, 139(42),* 2148-2152.

Gärtner, R., Gasnier, B.C.H., Dietrich, J.W., Krebs, B. & Angstwurm, W.A. (2002). Selenium supplementation in patients with autoimmune thyroiditis decreases thyroid peroxidase antibodies concentrations. *J Clin Endocrinol Metab, 87,* 1687-1691.

Ge, J.-F., Peng, L., Hu, C.-M. & Wu, T.-N. (2012). Impaired learning and memory performance in subclinical hypothyroidism rat model induced by hemi-thyroid electrocauterization. *J Neuroendocrinol, 24(6),* 953-961.

Ghenimi, N., Alfos, S., Redonnet, A., Higueret, P, Pallet, V. & Enderlin, V. (2010). Adult-onset hypothyroidism induces amyoidogenic pathway of amyoid precursor protein processing in the rat hippocampus. *J Neuroendocrinol, 22,* 951-959.

Grabe, H.J., Völzke, H., Lüdemann, J., Wolff, B., Schwahn, C., John, U., Meng, W. & Freyberger, H.J. (2005). Mental and physical complaints in thyroid disorders in the general population. *Acta Psychiatr Scand, 112; 286*-293.

Greicius, M.D., Srivastava, G., Reiss, A.L. & Menon, V. (2004). Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. *PNAS, 101(13),* 4637-4642.

Grozinsky-Glasberg, S., Fraser, A., Nahshoni, E., Weizman, A. & Leibovici, L. (2006). Thyroxine-triiodothyronine combination therapy versus thyroxine monotherapy for clinical hypothyroidism: meta-analysis of randomized controlled trials. *J Clin Endocrinol Metab, 91(7),* 2592-2599.

Hassan, W.A., Aly, M.S., Abdel Rahman, T. & Shahat, A.S. (2013). Impact of experimental hypothyroidism on monoamines level in discrete brain regions and other peripheral tissues of young and adult male rats. *Int J Devl Neurosci, 31,* 225-233.

Hautzinger, M., Bailer, M., Worall, H. & Keller, F. (1994). *Beck-Depressions-Inventar (BDI).* Bern: Hans Huber Verlag.

He, X.S., Ma, N., Pan, Z.L., Wang, Z.X., Li, N., Zhang, X.C., Zhou, J.N., Zhu, D.F. & Zhang, D.R. (2011). Functional magnetic resource imaging assessment of altered brain function in hypothyroidism during working memory processing. *Eur J Endocrinol, 164,* 951-959.

Henke, K., Buck, A., Weber, B. & Wieser, H.G. (1997). Human hippocampus establishes associations in memory. *Hippocampus, 7,* 249-256.

Hetzel, B.S. (2005). Towards the global elimination of brain damage due to iodine deficiency – the role of the international council for control of iodine deficiency disorders. *Int J Epidemiol, 34(4),* 762-764.

Jäncke, L. (2005). *Methoden der Bildgebung in der Psychologie und den kognitiven Neurowissenschaften.* Stuttgart: Kohlhammer.

Jaeschke, R., Guyatt, G., Gerstein, H., Patterson, C., Molloy, W., Cook, D., Harper, S., Griffith, L. & Carbotte R. (1996). Does treatment with l-thyroxine influence health status in middle-aged and older adults with subclinical hypothyroidism? *J Gen Intern Med, 11;* 744-749.

Janssen, O.E., Heufelder, A.E. & Mann, K. (2001). Kapitel 3, Schilddrüsenerkrankungen. In: D. Ganten & K. Ruckpaul (Eds.). *Endokrinopathien.* Heidelberg: Springer.

Joffe, R.T., Pearce, E.N., Hennessey, J.V., Ryan, J.J. & Stern, R.A. (2012). Subclinical hypothyroidism, mood, and cognition in older adults: a review. *Int J Geriatr Psychiatry, 28(2),* 111-118.

Johnson, L.Q., Hobson, V., Jenkins, M., Dentino, A., Ragain, R.M. & O'Bryant, S. (2011). The influence of thyroid function on cognition in a sample of ethnically diverse, rural-dwelling women: a project FRONTIER study. *J Neuropsychiatry Clin Neurosci, 23(2),* 219-222.

De Jong, F.J., den Heijer, T., Visser, T.J., de Rijke, Y.B., Drexhage, H.A., Hofman, A. & Breteler, M.M.B. (2006). Thyroid hormones, dementia, and atrophy of the medial temporal lobe. *J Clin Endocrinol Metab, 91(7),* 2569-2573.

De Jong, F.J., Peeters, R.P., den Heijer, T., van der Deure, W.M., Hofman, A., Uitterlinden, A.G., Visser, T.J. & Breteler, M.M.B. (2007). The association of polymorphisms in the type 1 and 2 deiodinase genes with circulating thyroid hormone parameters and atrophy of the medial temporal lobe. *J Clin Endocrinol Metab, 92,* 636-640.

De Jongh, R.T., Lips, P., van Schoor, N.M., Rijs, K.S., Deeg, D.J., Comijs, H.C., Kramer, M.H., Vandenbroucke, J.P. & Dekkers, O.M. (2011). Endogenous subclinical thyroid disorders, physical and cognitive function, depression, and mortality in older individuals. *Eur J Endocrinol, 165(4),* 545-554.

Jorde, R., Waterloo, K., Storhaug, H., Nyrnes, A., Sundsfjord, J. & Jenssen, T.G. (2006). Neuropsychological function and symptoms in subjects with subclinical hypothyroidism and the effect of thyroxine treatment. *J Clin Endocrinol Metab, 91(1),* 145-153.

Jørgensen, P., Langhammer, A., Krokstad, S. & Forsmo, S. (2014). Is there an association between disease ignorance and self-rated health? The HUNT study, a cross-sectional survey. *BMJ Open, 4,* e004962.

Kahaly, G.J., Dienes, H.P., Beyer, J. & Hommel, G. (1998). Iodide induces thyroid autoimmunity in patients with endemic goiter: a randomized, double-blind, placebo-controlled trial. *European Journal of Endocrinology, 139,* 290-297.

Kaya, M., Cermik, T.F., Bedel, D., Kutucu, Y., Tuglu, C. & Yigitbasi, Ö.N. (2007). Assessment of alterations in regional cerebral blood flow in patients with hypothyroidism due to Hashimoto's thyroiditis. *J Endocrinol Invest, 30,* 491-496.

Kong, W.M., Sheikh, M.H., Lumb, P.J., Freedman, D.B., Crook, M., Doré, C.J. & Finer, N. (2002). A 6-month randomized trial of thyroxine treatment in women with mild subclinical hypothyroidism. *Am J Med, 112;* 348-354.

Klaver, E.I., van Loon, H.C.M., Stienstra, R., Links, T.P., Keers, J.C., Kema, I.P., Muller Kobold, A.C., van der Klauw, M.M. & Wolffenbuttel, B.H.R. (2013). Thyroid hormone status and health-related quality of life in the LifeLines cohort study. *Thyroid, 23(9),* 1066-1073.

Kramer, C.K., von Mühlen, D., Kritz-Silversetin, D. & Barrett-Connor, E. (2009). Treated hypothyroidism, cognitive function, and depressed mood in old age: the Rancho Bernardo Study. *Eur J Endocrinol, 161,* 917-921.

Kratzsch, J., Schubert, G., Pulzer, F., Pfaeffle, F., Pfaeffle, R., Koerner, A., Dietz, A., Rauh, M., Kiess, W. & Thiery, J. (2008). Reference intervals for TSH and thyroid hormones are mainly affected by age, body mass index and number of blood leucocytes, but hardly by gender and thyroid autoantibodies during the first decade of life. *Clin Biochem, 41,* 1091-1098.

Krausz, Y., Freedman, N., Lester, H., Newman, J.P., Barkai, G., Bocher, M., Chisin, R. & Bonne, O. (2004). Regional cerebral blood flow in patients with mild hypothyroidism. *J Nucl Med, 45(10),* 1712-1715.

Kvetny, J., Ellervi, C. & Bech, P. (2014). Is suppressed thyroid-stimulating hormone (TSH) associated with subclinical depression in the Danish General Suburban Population Study? *Nord J Psychiatry,* epub ahead of print: doi: 10.3109/08039488.2014.972454.

Ladenson, P.W. (2002). Psychological wellbeing in patients. *Clin Endocrinol, 57,* 575-576.

Laurberg, P., Cerqueira, C., Ovesen, L., Rasmussen, L.B., Perrild, H., Andersen, S., Bülow Pedersen, I. & Carlé, A. (2010). Iodine intake as a determinant of thyroid disorders in populations. *Best Pract Res Clin Endocrinol Metab, 24,* 13-27.

Leyhe, T., Ethofer, T., Bretscher, J., Künle, A., Säuberlich, A.-L., Klein, R., Gallwitz, B., Häring, H.-U., Fallgatter, A., Klingberg, S., Sauer, R. & Müssig, K. (2013). Low performance in attention testing is associated with reduced grey matter density of the left inferior frontal gyrus in euthyroid patients with Hashimoto's thyroiditis. *Brain Behav Immun, 27(1)*, 33-37.

Leyhe, T., Müssig, K., Weinert, C., Laske, C., Häring, H.-U., Saur, R., Klingberg, S. & Gallwitz, B. (2008). Increased occurence of weaknesses in attention testing in patients with Hashimoto's thyroiditis compared to patients with other thyroid illnesses. *Psychoneuroendocrinology, 33,* 1432-1436.

Logothetis, N.K. (2008). What we can do and what we cannot do with fMRI. *Nature, 453,* 869-878.

Margulies, D.S., Böttger, J., Long, X., Lv, Y., Kelly, C., Schäfer, A., Goldhahn, D., Abbushi, A., Milham, M.P., Lohmann, G. & Villringer, A. (2010). Resting developments: a review of fMRI post-processing methodologies for spontaneous brain activity. *Magn Reson Mater Phy, 23,* 289-307.

McMillan, C., Bradley, C., Razvi, S. & Weaver, J. (2006). Psychometric evaluation of a new questionnaire measuring treatment satisfaction in hypothyroidism: the ThyTSQ. *Value Health, 9(2),* 132-139.

McMillan, C., Bradley, C., Razvi, S. & Weaver, J. (2008). Evaluation of new measures of the impact of hypothyroidism on quality of life and symptoms: the ThyDQoL and ThySRQ. *Value Health, 11(2),* 285-294.

Medici, M., Direk, N., Visser, W.E., Korevaar, T.I.M., Hofman, A., Visser, T.J., Tiemeier, H. & Peeters, R.P. (2014). Thyroid function within the normal range and the risk of depression: a population-based cohort study. *J Clin Endocrinol Metab, 99(4),* 1213-1219.

Meisinger, C., Ittermann, T., Wallaschofski, H., Meier, M., Below, H., Kramer, A., Döring, A., Nauck, M. & Völzke, H. (2012). Geographic variations in the frequency of thyroid disorders and thyroid peroxidase antibodies in persons without former thyroid disease within Germany. *Eur J Endocrinol, 167,* 363-371.

Miller, K.J., Parsons, T.D., Whybrow, P.C., van Herle, K., Rasgon, N., van Herle, A., Martinez, D., Silverman, D.H. & Bauer, M. (2006). Memory improvement with treatment of hypothyroidism. *Int J Neurosci, 116,* 895-906.

Montero-Pedrazueala, A., Fernández-Lamo, I., Alieva, M., Pereda-Pérez, I., Venero, C. & Guadaño-Ferraz, A. (2011).Adult-onset hypothyroidism enhances fear memory and upregulates mineralcorticoid and glucocorticoid receptors in the amygdala. *PlosOne, 6(10),* e26582.

Montero-Pedrazuela, A., Venero, C., Lavado-Autric, R., Fernández-Lamo, I., García-Verdugo, J., Bernal, J. & Guadaño-Ferraz, A. (2006). Modulation of adult hippocampal neurogenesis by thyroid hormones: implications in depressive-like behavior. *Mol Psychiatry, 11;* 361-371.

Moon, J.H., Park, Y.J., Kim, T.H., Han, J.W., Choi, S.H., Lim, S., Park, D.J., Kim, K.W. & Jang, H.C. (2014). Lower-but-normal serum TSH level is associated with the development or progression of cognitive impairment in elderly: Korean Longitudinal Study on Health and Aging (KLoSHA). *J Clin Endocrinol Metab, 99,* 424-432.

Nagamachi, S., Jinnouchi, S., Nishii, R., Ishida, Y., Fujita, S., Futami, S., Kodama, T., Tamura, S. & Kawai, K. (2004). Cerebral blood flow abnormalities induced by transient hypothyroidism after thyroidectomy. *Ann Nucl Med, 18(6),* 469-477.

Ott, J., Promberger, R., Kober, F., Neuhold, N., Tea, M., Huber, J.C. & Hermann, M. (2011). Hashimoto's thyroiditis affects symptom load and quality of life unrelated to hypothyroidism: a prospective case-control study in women undergoing thyroidectomy for benign goiter. *Thyroid, 21(2),* 161-167.

Owen, P.J.D. & Lazarus, J.H. (2003). Subclinical hypothyroidism: the case for treatment. *Trends Endocrinol Metab, 14(6),* 257-261.

Panicker, V., Evans, J., Bjøro, T., Åsvold, B.O., Dayan, C.M. & Bjerkese, O. (2009b). A paradoxical difference in relationship between anxiety, depression and thyroid function in subjects on and not on T4: findings from the HUNT study. *Clinical Endocrinology, 71,* 574-580.

Panicker, V., Saravanan, P., Vaidya, B., Evans, J., Hattersley, A.T., Frayling, T.M. & Dayan, C.M. (2009a). Common variation in the DIO2 gene predicts baseline psychological well-being and response to combination of thyroxine plus triiodthyronine therapy in hypothyroid patients. *J Clin Endocrinol Metab, 94,* 1623-1629.

Parle, J., Roberts, L., Wilson, S., Pattison, H., Roalfe, A., Haque, M.S., Heath, C., Sheppard, M., Franklyn, J. & Hobbs, F.D. (2010). A randomized controlled trial of the effect of thyroxine replacement on cognitive function in community-living elderly subjects with subclinical hypothyroidism: the Birmingham elderly thyroid study. *J Clin Endocrinol Metab, 95,* 3623-3632.

Parsaik, A.K., Singh, B., Roberts, R.O., Pankratz, S., Edwards, K.K., Geda, Y.E., Gharib, H., Boeve, B.F., Knopman, D.S. & Petersen, R.C. (2014). Hypothyroidism and risk of mild cognitive impairment in elderly persons. *JAMA Neurol, 71(2),* 201-207.

Pedersen, I.B., Knudsen, N., Carlé, A., Vejbjerg, P., Jørgensen, T., Perrild, H., Ovesen, L., Rasmussen, L.B. & Laurberg, P. (2011). A cautious iodization programme bringing iodine intake to a low recommended level is associated with an increase in the prevalence of thyroid autoantibodies in the population. *Clin Endocrinol, 75,* 120-126.

Pearce, E.N., Andersson, M. & Zimmermann, M.B. (2013). Global iodine nutrition: where do we stand in 2013? *Thyroid, 23(5),* 523-528.

Pfannenstiel, P., Hotze, L.A. & Saller, B. (1999). *Schilddrüsenkrankheiten - Diagnose und Therapie* (4th ed.). Berlin: Berliner Medizinische Verlagsanstalt.

Piga, M., Serra, A., Deiana, L, Loi, G.L., Satta, L., Di Liberto, M. & Mariotti, S. (2004). Brain perfusion abnormalities in patients with euthyroid autoimmune thyroiditis. *Eur J Nucl Med Mol Imaging, 31(12),* 1639-1644.

Pilhatsch, M., Marxen, M., Winter, C., Smolka, M.N. & Bauer, M. (2011). Hypothyroidism and mood disorders: integrating novel insights from brain imaging techniques. *Thyroid Research, 4 (suppl 1):* S3.

Pilhatsch, M., Winter, C., Nordström, K., Vennström, B., Bauer, M. & Juckel, G. (2010). Increased depressive behaviour in mice harboring the mutant thyroid hormone receptor alpha 1. *Behav Brain Res, 214;* 187-192.

Poldrack, R.A. & Gorgolewski, K.J. (2014). Making big data open: data sharing in neuroimaging. *Nat Neurosci, 17(11),* 1510-1517.

Pop, C.J., Maartens, LH., Leusink, G., van Son, M.J., Knottnerus, A.A., Ward, A.M., Metcalfe, R. & Weetman, A.P. (1998). Are autoimmune thyroid dysfunction and depression related? *J Clin Endocrinol Metab, 83(9),* 3194-3197.

Prummel, M.F. & Wiersinga, W.M. (2005). Thyroid peroxidase autoantibodies in euthyroid subjects. *Best Pract Res Clin Endocrinol Metab, 19(1),* 1-15.

Quidé, Y., Witteveen, A.B., El-Hage, W., Veltman, D.J. & Olff, M.(2012). Differences between effects of psychological versus pharmacologicaltreatments on functional and morphological brainalterations in anxiety disorders and major depressive disorder: asystematic review. *Neurosci Biobehav Rev, 36,* 626-644.

Quinque, E.M., Karger, S., Arélin, K., Schroeter, M.L., Kratzsch, J. & Villringer, A. (2014). Structural and functional MRI study of the brain, cognition and mood in long-term adequately treated Hashimoto's thyroiditis. *Psychoneuroendocrinology, 42*, 188-198.

Quinque, E.M., Villringer, A., Kratzsch, J. & Karger, S. (2013). Patient- reported outcomes in adequately treated hypothyroidism – insights from the German versions of ThyDQoL, ThySRQ and ThyTSQ. *Health Qual Life Outcomes 11;* 68.

Resta, F., Triggiani, V., Barile, G., Benigno, M., Suppressa, P., Giagulli, V.A., Guastamacchia, E. & Sabbà, C. (2012). Subclinical hypothyroidism and cognitive dysfunction in the elderly. *Endocr Metab Immune Disord Drug Targets, 12,* 260-267.

Roberts, C.G.P. & Ladenson, P.W. (2004). Hypothyroidism. *The Lancet, 363,* 793-803.

Samuels, M.H. (2010). Cognitive function in subclinical hypothyroidism. *J Clin Endocrinol Metab, 95(8),* 3611-3613.

Samuels, M.H., Schuff, K.G., Carlson, N.E., Carello, P. & Janowsky, J.S. (2007). Health status, mood, and cognition in experimentally induced subclinical hypothyroidism. *J Clin Endocrinol Metab, 92(7),* 2545-2551.

Saravanan, P., Chau, W.-F., Roberts, N., Vedhara, K., Greenwood, R. & Dayan, C.M. (2002). Psychological well-being in patients on 'adequate' doses of l-thyroxine: results of a large, controlled community-bases questionnaire study. *Clin Endocrinol, 57,* 577-585.

Schneider, F. & Fink, G.R. (Eds.). (2013). *Funktionelle MRT in Psychiatrie und Neurologie* (2nd ed.). Heidelberg: Springer Verlag.

Schraml, F.V. & Beason-Held, L.L. (2010). Technetium-99m ethyl cysteinate dimer (ECD) cerebral accumulation and symptom and sign severity during hypothyroidism. *Neuro Endocrinol Lett, 31(1),* 161-167.

Schraml, F.V., Goslar, P.W., Baxter, L. & Beason-Held, LL. (2011). Thyroid stimulating hormone and cognition during severe, transient hypothyroidism. *Neuro Endocrinol Lett, 32(3),* 279-285.

Singh, S., Modi, S., Bagga, D., Kaur, P. Shankar, L.R. & Khushu, S. (2013). Voxel-based morphometry analysis in hypothyroidism using diffeomorphic anatomic registration via an exponentiated lie algebra algorithm approach. *J Neuroendocrinol, 25,* 229-234.

Sperling, R.A., Chua, E., Cocchiarella, A., Rand-Giovannetti, E., Poldrack, R., Schacter, D.L. & Albert, M. (2003). Putting names to faces: successful encoding of associative memories activates the anterior hippocampal formation. *NeuroImage, 20,* 1400-1410.

Vanderpump, M. (2003). Subclinical hypothyroidism: the case against treatment. *Trends Endocrinol Metab, 14(6),* 262-266.

Vanderpump, M. (2011). The epidemiology of thyroid disease. *Br Med Bull, 99,* 39-51.

Vanderpump, M. & Tunbridge, W. M. G. (2002). Epidemiology and prevention of clinical and subclinical hypothyroidism. *Thyroid, 12(10),* 839-847.

Van de Ven, A., Muntjewerff, J.W., Netea-Meier, J., de Vegt, F., Ross, H.A., Sweep, F.C., Kiemeney, L.A., Vos, P.E., Buitelaar, J.K., Hermus, A.R., den Heijer, M. & Janzing, J.G. (2012a). Association between thyroid function, thyroid autoimmunity, and state and trait factors of depression. *Acta Psychiatr Scand,* 126(5), 377-384.

Van de Ven, A., Netea-Maier, J., de Vegt, F., Ross, H.A., Sweep, F.C., Kiemeney, L.A., Hermus, A.R. & den Heijer, M. (2012b). Is there a relationship between fatigue perception and the serum levels of thyrotropin and free thyroxine in euthyroid subjects? *Thyroid, 22(12),* 1236-1243.

Völzke, H., Ittermann, T., Albers, M., Friedrich, N., Nauck, M., Below, H. & Kramer, A. (2012). Five-year change in morphological and functional alterations of the thyroid gland: the study of health in Pomerania. *Thyroid, 22(7),* 737-746.

De Vries, M., Ouwendijk, R., Kessels, A., de Haan, M.W., Flobbe, K., Hunink, M.R.M., van Engelshoven, J.M.A. & Nelemans, P.J. (2005). Comparison of generic and disease-specific questionnaires for the assessment of quality of life in patient with peripheral arterial disease. *J Vasc Surg, 41,* 261-268.

Walsh, J.P., Ward, L.C., Burke, V., Bhagat, C.I., Shiels, L., Henley, D., Gillett, M.J., Gilbert, R., Tanner, M. & Stuckey, B.G. (2006). Small changes in thyroxine dosage do not produce measurable changes in hypothyroid symptoms, well-being, or quality of life: results of a double-blind, randomized clinical trial. *J Endocrinol Metab, 91,* 2624-2630.

Weishaupt, D., Köchli, V.D. & Marincek, B. (2009). Wie funktioniert MRI? (6. Ed.) Heidelberg: Springer Medizin Verlag.

Wekking, E.M., Appelhof, B.C., Fliers, E., Schene, A.H., Huyser, J., Tijssen, J.G.P. & Wiersinga, W.M. (2005). Cognitive functioning and well-being in euthyroid patients on thyroxine replacement therapy for primary hypothyroidism. *Eur J Endocrinol, 153,* 747-753.

Wheeler, S.M., McAndrews, M.P., Sheard, E.D. & Rovet, J. (2011). Visuospatial associative memory and hippocampal functioning in congenital hypothyroidism. *J Int Neuropsychol Soc, 18,* 1-8.

Wiersinga, W.M. (2009). Do we need still more trials on T4 and T3 combination therapy in hypothyroidism? *Eur J Endocrinol, 161,* 955-959.

Witthaus, E., Stewart, J. & Bradley, C. (2001). Treatment satisfaction and psychological well-being with insulin glargine compared with NPH in patients with Type 1 diabetes. *Diabet Med, 18(8),* 619–625.

Yin, J.J., Liao, L.M., Luo, D.X., Xu, K., Ma, S.H., Wang, Z.X., Le, H.B., Huang, R.R., Cai, Z.L. & Zhang, J. (2013). Spatial working memory impairment in subclinical hypothyroidism: an fMRI study. *Neuroendocrinology, 97,* 260-270.

Zettinig, G., Asenbaum, S., Fueger, B.J., Hofmann, A., Diemling, M., Mittlboeck, M. & Dudczak, R. (2003). Increased prevalence of subclinical brain perfusion abnormalities in patients with autoimmune thyroiditis: evidence of Hashimoto's encephalitis. *Clin Endocrinol, 59,* 637-643.

Zhu, D.F., Wang, Z.X., Zhang, D.R., Pan, Z.L., He, S., Hu, X.P., Chen, X.C., Zhou, J.N. (2006). fMRI revealed neural substrate for reversible working memory dysfunction in subclinical hypothyroidism. *Brain, 129,* 2923-2930.

VII Appendix

1 Supplemental Material, Quinque et al., 2014

Structural and functional MRI study of the brain, cognition and mood in long-term adequately treated Hashimoto's thyroiditis

Eva M. Quinque, Stefan Karger, Katrin Arélin, Matthias L. Schroeter, Jürgen Kratzsch and Arno Villringer

Supplemental Material

This material supplements but does not replace the content of the peer-reviewed paper published in Psychoneuroendocrinology

Methods

MRI acquisition parameters

T1-weighted images were acquired using a three-dimensional magnetisation-prepared rapid gradient echo sequence (sagittal orientation) with selective water excitation and linear phase encoding. The following imaging parameters were used: Inversion time (TI) 650 ms, repetition time (TR) 1.3 sec., repetition time of the gradient-echo kernel 9.1 ms, echo time (TE) 3.46 ms, flip angle 10°, field of view 256x240 mm^2, 2 averages. To avoid aliasing, oversampling was performed in read direction (head to foot). Reconstructed images were calculated using zero filling with 1x1x1 mm^3 voxel size.

The resting-state and event-related fMRI data were acquired using T2*-weighted gradient echo-planar image (EPI) pulse sequences with the following parameters:

300 volumes, TR 2.0 sec., TE 30 ms, flip angle 90°, 30 slices, voxel resolution 3 mm * 3 mm * 4 mm. During the event-related fMRI session, participants performed an associative memory encoding task adapted from Sperling et al., (2003) presented with Presentation software (Neurobehavioral Systems, Inc., version 12.7) on a back-projection screen viewed via a mirror. Participants viewed black-and-white photographs of faces (Jäger et al., 2005; http://psydok.sulb.uni-saarland.de/volltexte/2005/505/) with a first name written underneath and were asked to remember each person's name. They had to decide whether they felt the name fitted the face or not by a button press. Participants viewed a fixation cross between trials. Participants viewed 90 encoding trials randomly interleaved with 30 nullevents where they were shown a fixation cross for the trial duration of eight seconds. After five minutes rest, participants performed the retrieval task in the scanner while structural scans were acquired. During retrieval they viewed all faces again, this time paired with the learned name and a distractor name paired with another face during encoding. Participants had to choose the correct name by a button press and reported whether they were sure or unsure about their response. There was no time limit to ensure that all trials were responded to. Face-name pairings were randomised for each participant and different sets were used at the two sessions.

Grey matter data analysis

Images were bias-field corrected, segmented and registered to standard Montreal Neurological Institute (MNI) space using rigid-body transformation and the unified segmentation approach (Ashburner and Friston 2005). Subsequently, images were smoothed with a Gaussian kernel of 8 mm^3 full width at half maximum (FWHM). Resulting grey matter images were averaged across the two time points and entered into group level analyses. All voxels with a minimum grey matter density probability of

0.2 were included in the analyses. The exact coordinates of the regions reported in Bauer et al., (2009) did not lie within grey matter for all subjects and 9 mm spheres (27 voxels) were thus created around the described areas instead. Coordinates for the areas were taken from the Harvard-Oxford Structural Atlas provided by Functional magnetic resonance imaging of the brain Software Library (FSL), the resulting regions of interest (ROIs) lying within grey matter for all subjects. The six ROIs are the right hippocampus (MNI coordinates x=30, y=-24 z=-12), left hippocampus (-30 -27 -12), right amygdala (27 0 -21), left amygdala (-24 -3 -18), posterior cingulate cortex (0 -27 36) and subcallosal cortex (0 24 -12).

Resting-state fMRI data analysis

The preprocessing steps comprised three-dimensional motion correction, time series despiking, 6 mm FWHM spatial smoothing, four-dimensional mean-based intensity normalisation, band-pass temporal filtering (0.01-0.1 Hz), removing linear and quadratic trends and regressing out eight nuisance signals (white matter, cerebrospinal fluid and six motion parameters). No global signal regression was performed to avoid spurious negative correlations driving group comparisons. The output of these preprocessing steps was one 4-dimensional residual functional volume for each participant. The normalisation of the functional volume was performed via linear normalisation to MNI space using the FSL Linear Image Registration Tool and the individual T1-weighted image as a prior.

Seed-based resting-state functional connectivity analysis calculates the correlation between the seed regions and each other voxel in the brain. The resulting connectivity maps per subject were subsequently averaged across the two time points and entered into separate group comparisons to identify regions of altered connectivity to the six seed regions. Significance level was set to $p<0.0083$ on the

family-wise error corrected cluster size level to account for the use of six seed regions for group and correlation analyses.

An explorative functional connectivity analysis was performed with those areas as seeds that revealed a significant correlation between grey matter density and mood in the patient group. This was done in order to gain information about involved networks in addition to the result on isolated structures available with the VBM analysis. Seeds were extracted using Marsbar, a toolbox for SPM8 (http://marsbar.sourceforge.net). Connectivity maps of the patient group were entered into a onesample t-test controlled for age and sex. An FWE-corrected voxel-level threshold of $p<0.005$ was chosen for illustration purposes and functional networks were descriptively compared with networks reported in the literature (Beckmann et al., 2005; Fox and Raichle 2007; Wang et al., 2012).

Event-related fMRI data analysis

Task-based fMRI data was preprocessed using SPM8 running on Matlab. Data were slice-time corrected, realigned, corrected for field inhomogeneities (unwarping) and normalised to MNI space using the unified segmentation approach (Ashburner and Friston, 2005). EPI deformations were corrected using a fieldmap scan acquired separately during the scanning session. An 8 mm Gaussian kernel was used for smoothing and a high-pass filter of 128 Hz was used to account for slow signal drifts.

Results

The right postcentral gyrus was associated with a network resembling a sensory-motor network (Beckmann et al., 2005; Figure 6d) comprising pre- and postcentral gyrus and midcingulate areas. The left superior frontal gyrus revealed an executive control network including mainly frontal areas (Beckmann et al., 2005; Figure 6f). The

left cuneus was associated with a visual network consisting of mainly occipital areas (Beckmann et al., 2005; Figure 6a) and the middle temporal gyrus belonged to the default mode network including posterior and anterior cingulate cortices, medial prefrontal cortex and inferior parietal cortex (Wang et al., 2012, see Figure S1). The left temporal pole did not reveal a meaningful network as it only correlated with regions directly adjacent to the seed region and its contralateral equivalent.

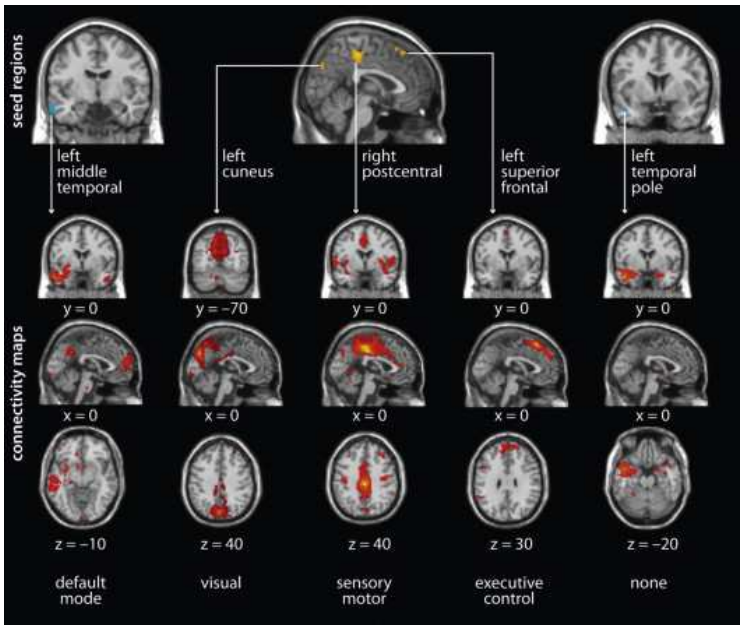

Figure S1: Resting-state networks of areas showing an association between grey matter density and mood in the patient group. Connectivity maps are shown on coronal, sagittal and axial slices oriented according to neurological convention. Labels of resembling networks as described by Beckmann et al., 2005 or Wang et al., 2012 are shown next to the connectivity maps.

2 Declaration of Authenticity

Eigenständigkeitserklärung

Erklärung über die eigenständige Abfassung der Arbeit

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Ich versichere, dass Dritte von mir weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, und dass die vorgelegte Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt wurde. Alles aus anderen Quellen und von anderen Personen übernommene Material, das in der Arbeit verwendet wurde oder auf das direkt Bezug genommen wird, wurde als solches kenntlich gemacht. Insbesondere wurden alle Personen genannt, die direkt an der Entstehung der vorliegenden Arbeit beteiligt waren.

Leipzig, März 2015 Eva Quinque

3 Curriculum Vitae

Personal Data

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Date & place of birth	25.04.1985 in Pegnitz, Bavaria
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Education

since 10/2010	Department of Neurology, Max-Planck-Institute for Human Cognitive and Brain Sciences (MPI), Leipzig PhD: *Brain, mood and cognition in hypothyroidism* (A. Villringer)
10/2007 – 09/2008	Department of Experimental Psychology, University of Cambridge, GB Master of Philosophy in the Biological Sciences: *Individual differences in iconic memory* (J.D. Mollon)
10/2004 – 09/2007 & 10/2008 – 09/2010	Department of Psychology, University of Leipzig Diplom (1.0): *Social cognition and executive functions in vascular cognitive disorder* (S. Frisch, M.L. Schroeter)
06/2003	Abitur Ratsgymnasium Osnabrück (1.2)

Work experience

since 12/2014	Scientific staff member at the Department of Neurology (A.Villringer), MPI
04/2009 – 09/2010	Student assistant at the Department of Neurology (A. Villringer), MPI
10/2009 – 12/2009	Intern at the Day Clinic for Cognitive Neurology, University Clinic Leipzig
01/2009 – 09/2009	Intern at the Memory Consultation Unit, University Clinic Leipzig
05/2005 – 09/2007	Student assistant at the Department of Cognitive Neurology (D.Y. von Cramon), MPI

Scholarships

10/2010 – 11/2014	Max Planck Society
10/2007 – 07/2008	DAAD and Kurt Hahn Trust
04/2005 – 09/2010	Studienstiftung des deutschen Volkes

4 List of publications

2014

*__Quinque, E.M.__, Karger, S., Arélin, K., Schroeter, M.L., Kratzsch, J. & Villringer, A. (2014). Structural and functional MRI study of the brain, cognition and mood in long-term adequately treated Hashimoto's thyroiditis. *Psychoneuroendocrinology, 42*, 188-198.

Frisch, S., **Quinque, E.M.** & Schroeter, M.L. (2014). Kognitive Funktionsstörungen bei früher zerebraler Mikroangiopathie (Cognitive deficits in early small vessel disease). *Neuro aktuell: Informationsdienst für Neurologen & Psychiater, 27(8),* 17.

Schäfer, A., **Quinque, E.M.**, Kipping, J., Arélin, K., Roggenhofer, E., Frisch, S., Villringer, A., Mueller, K. & Schroeter, M.L. (2014). Early small vessel disease affects frontoparietal and cerebellar hubs in close correlation with clinical symptoms: A resting-state fMRI study. *Journal of Cerebral Blood Flow and Metabolism 34(7*), 1091-1095.

2013

*__Quinque, E.M.__, Villringer, A., Kratzsch, J. & Karger, S. (2013). Patient- reported outcomes in adequately treated hypothyroidism−− insights from the German versions of ThyDQoL, ThySRQ and ThyTSQ. *Health Qual Life Outcomes 11;* 68.

2012

Quinque, E.M., Arélin, K., Dukart, J., Roggenhofer, E., Streitbürger, D.P., Villringer, A., Frisch, S., Mueller, K. & Schroeter, M.L. (2012). Identifying the neural correlates of executive functions in early cerebral microangiopathy: A combined VBM and DTI study. *Journal of Cerebral Blood Flow and Metabolism 32(10)*, 1869-1878.

* the two publications marked with an asterix form the present cumulative dissertation

Conference contributions

2015

Quinque, E.M., Karger, S., Arélin, K., Schroeter, M.L., Kratzsch, J. & Villringer, A. (2015). Brain, mood and cognition during treatment initiation in mild hypothyroidism. Poster accepted for presentation at 17th European Congress of Endocrinology (ECE), Dublin, Ireland.

2013

Schäfer, A., **Quinque, E.M.**, Kipping, J., Arélin, K., Roggenhofer, E., Frisch, S., Villringer, A., Müller, K. & Schroeter, M.L. (2013). Central hubs affected by early small vessel disease. Poster presented at 19th Annual Meeting of the Organization for Human Brain Mapping (OHBM), Seattle, WA, USA.

2010

Weig, E.M., Arélin, K., Semler, V., Spengler, S., Villringer, A. & Schroeter, M.L. (2010). Social cognition is impaired besides executive functions in cerebral microangiopathy. Poster presented at Annual Meeting of German Society for Psychiatry, Psychotherapy and Neurology (DGPPN), Berlin, Germany.

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