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Experimentally induced subclinical hypothyroidism causes decreased functional connectivity of the cuneus: A resting state fMRI study

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ABSTRACT

Objective: The aim of this study was to experimentally evaluate the effects of subclinical mild hypothyroidism on brain network connectivity as determined by resting state fMRI (rsfMRI) which serves as a proxy for global changes in brain function.

Methods: Fifteen otherwise healthy patients with complete hypothyroidism under stable, long term levothyroxine substitution volunteered for the study. They reduced their pretest levothyroxine dosage by 30% for 52–56 days. Basally and after partial levothyroxine withdrawal, rsfMRI along with a neuropsychological analysis was performed. RsfMRI was subjected to graph-theory-based analysis to investigate whole-brain intrinsic functional connectivity.

Results: The desired subclinical hypothyroidism was achieved in all subjects. This was associated with a significant decrease in resting-state functional connectivity specifically in the cuneus (0.05 FWE corrected at cluster level) which was mainly caused by a weaker functional connectivity to the cerebellum and regions of the default mode network, i.e. the medial prefrontal cortex, the precuneus and the bilateral angular gyri. The decrease in cuneus connectivity was correlated to the increase in TSH serum levels. A working memory task showed a slightly longer reaction time and less accuracy after partial levothyroxine withdrawal.

Conclusion: Even short-term partial levothyroxine partial withdrawal leads to deficits in working memory tasks and to a weaker integration of the cuneus within the default mode network.

1. Introduction

The impact of thyroid hormone status on higher cognitive functions is well known. Very profound cognitive changes have been described particularly in hypothyroidism during prenatal development and into early childhood (Zoeller and Rovet, 2004) which attenuate towards adulthood. But even in adults an underactive thyroid alters cognitive function with particular memory deficits being most prominent (Beydoun et al., 2015). The mildest form of hypothyroidism, subclinical hypothyroidism, is characterized by normal peripheral thyroxine and triiodothyronine levels but and an already increased plasma thyroid-stimulating hormone (TSH) concentration (Cooper and Biondi, 2012). Whether subclinical hypothyroidism also induces cognitive impairments is currently under debate (Pasqualetti et al., 2015) but the majority of studies suggests deficits in executive functions and working memory (Samuels, 2014).

Few prior neuroimaging studies have analyzed subclinical hypothyroidism (Yin et al., 2013; Zhu et al., 2006). Prior neuroimaging studies have shown changed functional activity in subclinical hypothyroid patients of bilateral frontal areas, parietal lobes, anterior cingulate cortex, thalamus and supplementary motor area (Yin et al., 2013). Additionally, the study could show that levothyroxine treatment was able to reverse the altered neural activity (Yin et al., 2013). Another study (Zhu et al., 2006) showed that frontal areas were affected by subclinical hypothyroidism, explaining executive dysfunctions experienced by these patients. The study (Zhu et al., 2006) was also able to show altered working memory ability using the n-back task in subclinical hypothyroid patients, suggesting that patients with subclinical

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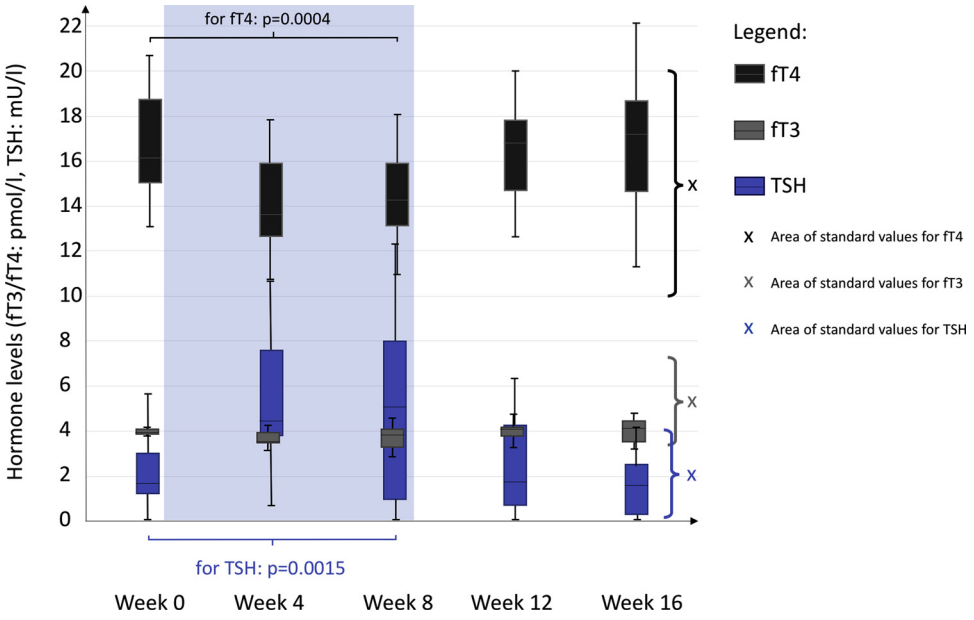

Fig. 1. Thyroid hormone levels of subjects, excluding two subjects with excessive head movement. Median and standard deviations are stated. Significant results for fT4 and TSH levels are stated, no significant results for fT3-levels. Blue-shaded area represents period of reduced levothyroxine dosage by 30% (between week 0 and week 8). Standard values for fT4 was 10-20 pmol/l, for fT3 3.4–7.2 pmol/l and for TSH 0.3-4.0 mU/l. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

hypothyroidism would benefit from clinical treatment.

Only one prior study has examined subclinical hypothyroid patients using resting state MRI (Kumar et al., 2018). Kumar et al. (2018) have shown an altered functional connectivity within the somato-motor network and right fronto-parietal attention network, both possibly contributing to mild motor, attention, executive and working memory impairments experienced by some subclinical hypothyroid patients. Short-term hyperthyroidism resulted in an increased functional connectivity between temporal lobes and cognitive control network (Göttlich et al., 2015a). All these studies show that hypothyroid/subclinical hypothyroid patients may experience neurocognitive deficits due to abnormal alterations of brain networks, functional and structural changes. Hypothyroidism has been associated with alterations of functional connectivity in frontoparietal nertwork, medial visual network and motor network in overt hypothyroid patients (Shin et al., 2016; Singh et al., 2015). It has been shown that patients with new-onset untreated hyperthyroidism showed lower amplitude of low-frequency fluctuation in the posterior cingulate cortex, increased functional connectivity in insula and anterior cerebellum, as well as decreased functional connectivity in prefrontal cortex, temporal gyrus and posterior cingulate cortex (Liu et al., 2017). The study group (Liu et al., 2017) was additionally able to show a correlation of improvement of regional functional connectivity with efficacy of treatment.

In the present study we experimentally rendered otherwise healthy patients under long-term substitution because of a severely underactive thyroid subclinically hypothyroid by reducing their long-term levothyroxine dose by 30% for eight weeks. We evaluated the effects on resting-state networks and applied a graph-theory-based analysis to identify brain regions of altered functional connectivity. The degree centrality served as a measure of voxel-wise whole-brain connectivity and is defined as the number of connections to other voxels within the brain. The degree centrality is a very sensitive marker to identify regions of altered connectivity in a data-driven approach and was successfully used in previous studies investigating Parkinson's disease (Göttlich et al., 2013), Alzheimer's disease (Buckner et al., 2009), bilateral vestibular failure (Göttlich et al., 2014) and obsessive compulsive disorder (Beucke et al., 2013; Göttlich et al., 2015b). Our study group has recently used the same method to analyze the effects of experimentally induced thyrotoxicosis (250 µg levothyroxine p.o./day over eight weeks in healthy male volunteers) on intrinsic brain connectivity (Göttlich et al., 2015a). Induced thyrotoxicosis led to an increased resting-state functional connectivity in the rostral temporal lobes, which was caused by an increased functional connectivity to the cognitive control network. This increased connectivity may facilitate prefrontal control over limbic areas and may therefore underlie the successful use of thyroid hormones as an augmentation therapy for depression.

We now present the companion study investigating the effects of experimentally induced subclinical hypothyroidism. Our rationale to perform this study was to evaluate changes in comparison to our prior experimentally induced hyperthyroidism study. To the best of our knowledge, this is the first study published examining subjects after experimentally induced hypothyroidism. This is especially important, since there is still a debate in the literature whether subclinical hypothyroidism leads to cognitive changes, showing that further studies are needed. We hypothesized that mild hypothyroidism may affect functional connectivity, most likely in the same regions altered in hyperthyroidism.

2. Materials and methods

2.1. Ethics statement

All procedures were approved by the ethical committee of the University of Lübeck. The study was performed in agreement with the Declaration of Helsinki. All subjects gave their written informed consent prior to participation.

2.2. Subjects

Fifteen right-handed otherwise healthy patients (13 women; mean age of 37.92 years (range 19–61 years; mean BMI 24.05 ± 3.0) with severely underactive thyroid function under stable long-term levothyroxine replacement therapy to euthyroidism (stable for at least 3 months) volunteered for the study. Hormone blood levels were collected prior to enrollment in this study to confirm euthyroid state. Thirteen subjects suffered of Hashimoto thyroiditis, two subjects of Graves' disease with 1 subject thyroidectomized and 1 radioablated with radioactive iodine). All subjects were screened for general health, drug abuse, medication, mood and cognitive disorders. A neuroradiologist determined a normal structural cerebral MRI for all subjects.

Two subjects (one male and one female) had to be excluded due to head movements during MR-imaging. The changes in TSH and thyroid hormone levels are given in Fig. 1.

2.3. Experimental design

Each subject took part in two identical scanning sessions. For the first session, all subjects were analyzed in the euthyroid state before reduction of their standard long-term levothyroxine dose. In between sessions, the subjects reduced their usual levothyroxine dosage by 30% for a period of 54–56 days. At the end of this period still under the reduced dose a second scanning session was performed. TSH and thyroid hormone levels were collected and neuropsychological analysis was performed at each scanning session. For safety reasons thyroid hormone status was additionally assessed after four weeks. The functional MRI data was acquired during a resting-state block of 6 min duration. Subjects were instructed to neither engage in cognitive nor motor activity and to keep their eyes closed. The MRI measurements were conducted in the afternoon.

2.4. Psychological test analysis

The n-back task (Owen et al., 2005) was performed to analyze changes in working memory. It consists of three conditions (0-back task, 1-back task, 2-back task). A series of letters (both upper and lower cases letters) is shown to the subjects. In the 0-back task condition, the subjects were supposed to tag a prior reported letter. In the 1-back task condition, the subjects were asked to tag a letter, if the same letter was presented one letter before. In the 2-back task condition, the subjects had to tag a letter, if it was presented two letters before. The subjects were required to temporarily store and delete the letters in their memory, while letters were shown continually to them. Results of this task in all subjects including those with head movements during rsfMRI have been published elsewhere (Göbel et al., 2018).

2.5. MRI acquisition and processing

Structural and functional MRI images were recorded on a Philips Achieva 3-T scanner (Philips Healthcare, the Netherlands) equipped with a standard 8-channel phase array head coil. A total of N = 178 functional images were acquired using a T2*-weighted single-shot gradient-echo echo-planar imaging (EPI) sequence sensitive to blood oxygen level dependent (BOLD) contrast using the following parameters: repetition time TR = 2000 ms; echo time TE = 28 ms; $3 \times 3\,mm^2$ in-plane resolution; in-plane field of view 192 mm; slice thickness 3 mm, 40 axial slices, flip angle 80° and SENSE factor R = 1.8. High resolution structural images were obtained applying a T1-weighted 3D turbo gradient-echo sequence with SENSE (image matrix 240 × 240; 180 slices; $1 \times 1 \times 1\ mm^3$ spatial resolution).

Preprocessing was performed using the SPM12 software package (University College London, Wellcome Trust Centre for Neuroimaging (http://www.fil.ion.ucl.ac.uk/spm). The first 10 images of each dataset were discarded to allow for magnetization equilibrium and for the subjects to adjust to the environment. The preprocessing included the following steps: (i) Correction for differences in the image acquisition time between slices; (ii) a six parameter rigid body spatial transformation to correct for head motion during data acquisition; (iii) co-registration of the structural image to the mean functional image; (iv) grey and white matter segmentation, bias correction and spatial normalization of the structural image to a standard template (Montreal Neurological Institute); (v) In order to reduce the influence of motion and unspecific physiological effects, a regression of nuisance variables from the data was performed. Nuisance variables included white matter and ventricular signals and the six motion parameters determined in the realignment procedure. (vi) spatial normalization of the functional images using the normalization parameters estimated in the previous preprocessing step and resampling to $3 \times 3 \times 3\,mm^3$; (vii) spatial smoothing with a 6 mm full width half maximum Gaussian kernel. (viii) A temporal bandpass filter was applied to all voxel time series (0.01 Hz < f < 0.08 Hz).

The six realignment parameters, i.e. three displacements and three elementary rotations with respect to the first image in the EPI series, were used as an estimator for the head motion. The displacements with respect to the first image of the series were required to be smaller than 3.0 mm (minimum to maximum) and the individual rotations smaller than 3.0°. Two subjects had to be excluded according to these criteria.

2.6. Voxel degree centrality maps

Voxel-wise degree centrality maps (voxel-wise whole brain connectivity maps) were calculated by correlating the temporal BOLD signal fluctuation of each voxel with all other voxels in the brain and counting the number of connections above a certain threshold. As a measure for the temporal correlation, we computed the zero-lag Pearson's linear correlation coefficient r. The individual correlation coefficients were entered into an N × N adjacency matrix where N is the number of voxels within the brain mask. The brain mask covered the whole brain including grey matter, white matter and ventricles. The voxel network matrix was thresholded by $r > 0.25$ suppressing random correlations. This results in a binary undirected network matrix d_{ij}. The voxel degree D_i was derived from the network matrix as follows:

$$D_i = \sum_{j=1}^{N} d_{ij}$$

The degree maps were z-transformed:

$$z_i = \frac{D_i - \bar{D}}{\sigma_D}\ (i = 1...N)$$

Here, $\bar{D}$ denotes the mean degree and σ_D the standard deviation. This transformation ensured that the degree centrality maps were comparably scaled and could be averaged and compared across subjects.

Seed-based connectivity analyses were performed to investigate the target regions of altered intrinsic functional connectivity, i.e. degree centrality. The time courses of all voxels within a particular seed region were averaged and connectivity maps were calculated by correlating the mean time course to all voxel time courses within a brain mask. Correlations were computed using the Pearson product moment formula. A Fisher z-transform was applied to all correlation maps prior to the statistical analysis. This yielded connectivity maps for each subject and both time points. A paired t-test was carried out to identify regions of altered connectivity.

2.7. Statistical analysis

Differences in the voxel-wise degree centrality were investigated applying a paired t-test. Statistical images were assessed for cluster-wise significance using a cluster-defining threshold of p = 0.001. A topological false discovery rate (FDR) procedure was used to correct for multiple comparisons (Chumbley et al., 2010). The analysis was performed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/). The statistical analysis of demographic, clinical and behavioural data was performed using Matlab®. If not stated otherwise we report the mean and standard deviation of the data.

3. Results

3.1. Thyroid hormone levels

Partial reduction of the levothyroxine dosage lead to a significant increase of TSH above the upper reference levels (p = 0.0015) and to significant decrease of fT4 (p = 0.0004) which nevertheless remained within the normal reference range (see Fig. 1). Thyroid hormone levels are stated below for all included subjects (see Fig. 1).

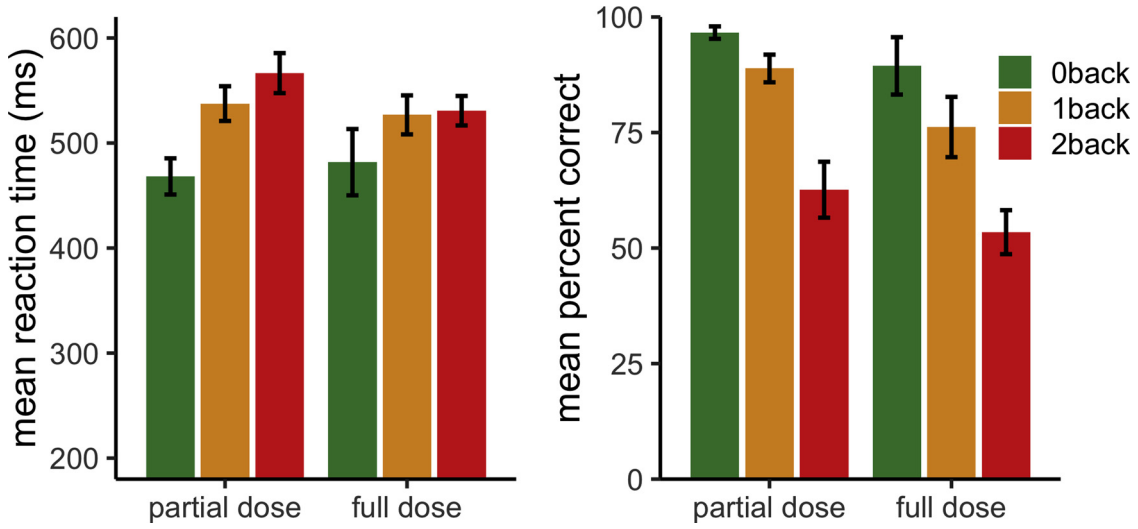

Fig. 2. Reaction times and performance accuracy in the n-back working memory task. Full levothyroxine dose vs partial levothyroxine dose showing significant changes in the 0-back task condition. For statistical analysis see Section 3.3.

3.2. Clinical symptoms

All subjects were evaluated by an experienced endocrinologists and showed no clinically detectable changes in symptoms or signs due to treatment. Symptoms evaluated included anxiety, perspiration, physical indisposition, restlessness, formication, trembling, hunger, heart palpitation, blurry vision, concentration, thirst, anger, headache, fullness, nausea, sadness, breathing difficulties, joy, tiredness, dizziness, nervousness, appetite, itchiness, weakness, warmth, activity and bloating.

3.3. Working memory

Both mean reaction time and mean correct responses were measured for n-back task (see Fig. 2). Induction of subclinical hypothyroidism prolonged reaction times. For the reaction time analysis, a repeated measures ANOVA showed a significant main effect for task difficulty ($F(2,24) = 17.78$, $p < 0.001$). However, when analyzing reaction time, there was no significant effect for thyroid state ($F(1,12) = 0.37$, $p = 0.2$) nor a task difficulty x thyroid state interaction ($F(1,12) = 1.57$, $p = 0.22$). For the analysis evaluating mean correct responses, ANOVA showed significant results for both task difficulty ($F(2,24) = 27.55$, $p < 0.001$) and thyroid state main effect ($F(1,12) = 7.11$, $p = 0.02$), but no significant interaction ($F(2,24) = 0.15$, $p = 0.85$).

3.4. Imaging results

Following intervention subjects showed a significantly (paired *t*-test; 0.05 FDR corrected at the cluster level) decreased whole-brain functional connectivity, i.e. degree centrality, in the cuneus (MNI coordinates of highest t-value in the cluster: x = 3, y = -78, z = 27 mm; peak t-value: 6.76; cluster size: 52 voxel; FWE corrected p-value: 0.028). The result is depicted in Fig. 3A. The relative change in degree centrality within the cuneus was significantly correlated with the relative change in TSH serum levels (Pearson's rho = −0.54; $p = 0.035$; one-tailed), i.e. the stronger the increase in TSH levels the stronger the decrease in cuneus whole-brain connectivity. For one male subject no TSH measurement was available. The correlation is thus based on 12 female subjects only as shown in Fig. 3A. Performing a partial correlation controlling for age did not change the result (rho = -0.53; $p = 0.045$).

Fig. 3B shows which brain regions are driving the effect of decreased cuneus connectivity ($z > 2.5$). We identified regions within the cerebellum and regions comprising the default mode network, i.e. the precuneus, the medial prefrontal cortex and the bilateral angular gyrus. Note, that this is a purely qualitative analysis as the result is biased due to the data driven choice of the seed-ROI.

The maximum head motion in our selected sample was 2.5 mm for both resting-state sessions. The mean head motion was 1.9 + -0.4 mm and 1.8 + -0.4 mm before and after the intervention, respectively. We conclude that there is no significant difference in head motion between the two sessions. Furthermore, the change in head motion between the two sessions was not correlated with the change in degree centrality (rho = 0.01; $p = 0.97$).

4. Discussion

We aimed to evaluate the effects of subclinical hypothyroidism of 8 weeks duration on the adult brain under highly controlled experimental conditions in otherwise healthy subjects. Under these strict conditions subtle changes in resting state connectivity were detected, possibly explaining the known cognitive effects of thyroid hypofunction. Underactive thyroid function clearly demonstrated biochemically was indeed subclinical as none of the subjects report significant changes in clinical symptoms or signs as assessed by an endocrinologist but they did show significantly less correct responses in a working memory task. Both findings, the lack of symptoms and signs as the impairment in working memory are in accordance to previous reports (Samuels, 2014; Samuels et al., 2007; Zhu et al., 2006).

4.1. Decreased connectivity for partial levothyroxine withdrawal

When subjects were tested under partial levothyroxine withdrawal a lower degree centrality in the cuneus was found in comparison to full dose replacement. This decrease was correlated to the increase in TSH blood serum levels. This is in concordance to previous literature, showing that serum TSH levels were inversely correlated to cerebral blood flow and cerebral glucose metabolism (Marangell et al., 1997). Previous reports suggest a correlation of thyroid hormone levels with cerebral glucose metabolism (Schreckenberger et al., 2006). Marangell et al. (1997) supported the lack of correlation between serum fT3/4 levels to cerebral blood flow in our study. This contrasts to a significant and inverse correlation between TSH and blood flow in the cuneus. Such correlation is questionable due to the small cohort tested and the pulsatile nature of TSH secretion but is supported by a comparable

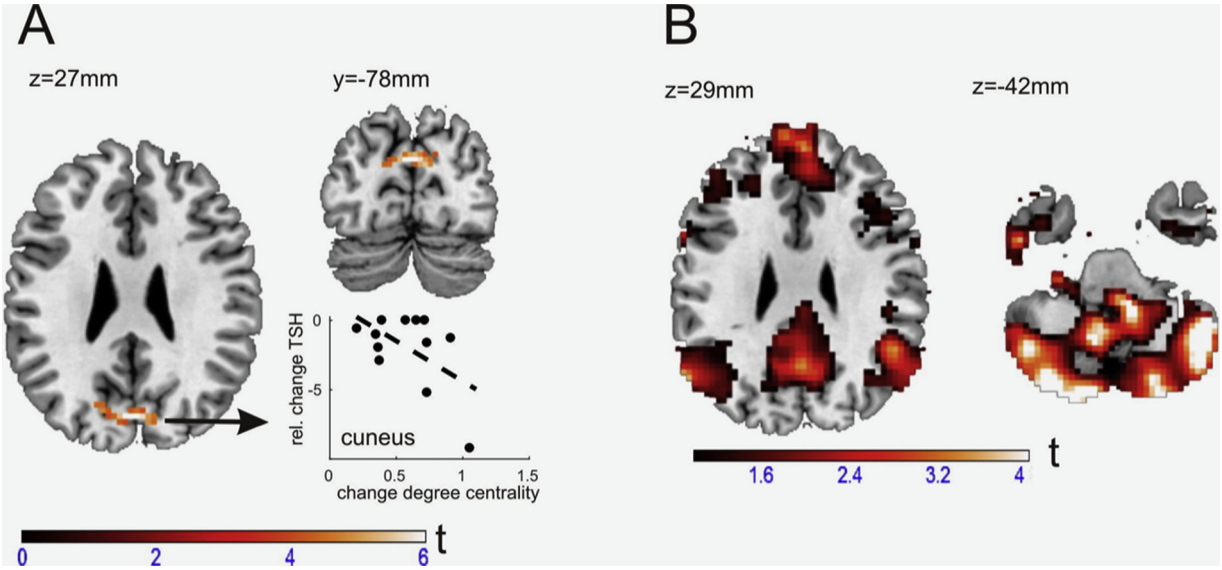

Fig. 3. A) Significant decrease in degree centrality in the bilateral cuneus under partial levothyroxine dose (topological FWE correction $p < 0.05$). Also shown is the relative change in degree centrality versus the relative change in TSH levels within the cuneus cluster. B) Map of aberrant cuneus connectivity showing the regions which were most strongly contributing to the effect of decreased degree centrality.

relation reported in two previous independent studies (Marangell et al., 1997; Schreckenberger et al., 2006)

The data thus fit to the assumption that the cuneus represents as a sensible target area in thyroid dysfunction. Regional cerebral blood flow has been shown to be lowered in mild hypothyroid patients in comparison to controls in inter alia the cuneus (Krausz et al., 2004; Nagamachi et al., 2004). In addition, in overtly hypothyroid patients glucose metabolism in the cuneus is reduced and inversely correlated to TSH (Constant et al., 2001). Furthermore, the cerebral blood flow in inter alia the cuneus has been shown to be reduced in patients with Alzheimer's disease suffering of subclinical hypothyroidism, but not in the same patient group with normal thyroid function (Haji et al., 2015). Finally, a lower functional integrity was detected using resting state fMRI in areas of the primary visual cortex, including the cuneus when hypothyroid patients were compared to controls (Singh et al., 2015). The cuneus is known to be involved in basic visual processing and is modulated by extra-retinal effects, including working memory, attention and reward expectation (Rosen et al., 2018). Interestingly, children suffering from early treated congenital hypothyroidism are known to suffer from difficulties in visuospatial memory tasks (Blasi et al., 2009). It therefore seems possible that these disturbances in visuospatial memory tasks are influenced by changes of the cuneus.

The lower degree centrality which we observed in the cuneus was mainly driven by weaker connectivity to brain regions comprising the default mode network, i.e. the precuneus, bilateral angular gyri and the medial frontal cortex, and posterior cerebellum (see Fig. 3). Resting state networks of the human cerebellum have been described in detail before (Bernard et al., 2012; Buckner et al., 2011), showing a broad distinction between the anterior and posterior cerebellum (Bernard et al., 2012). Lobules of the anterior cerebellum are correlated with motor cortical regions, while lobules of the posterior cerebellum are usually correlated with prefrontal and parietal cortices (Bernard et al., 2012). The posterior cerebellum appears to be closer connected to cognitive-relevant areas like the medial frontal cortex (Bernard et al., 2012), but lesion studies also show that damage in the posterior part (in contrast to the other parts of the cerebellum) specifically leads to cognitive and affective deficits (Buckner et al., 2011; Koziol et al., 2014). Additionally, in rodents direct effects of thyroid hormones on the cerebellum have been described. Absence of thyroid hormones during development results in a significantly decreased differentiation of cerebellar Purkinje cells (Morte et al., 2002). Hypothyroid mice show a significant delay of migrating granular cells from the external germinal layer to the internal layer in the cerebellum (Morte et al., 2002). The expression pattern of thyroid hormone receptors fit to these functional considerations with high expression in the cerebellum during development but very low expression in the rest of the brain (Williams, 2008). When we were studying the reverse situation of an experimentally induced hyperthyroidism in healthy volunteers for as well 52–56 days, functional activations using fMRI (Göbel et al., 2016a), increased perfusion (Göbel et al., 2016b) and an increase of grey matter volume (Göbel et al., 2015) of the posterior cerebellum could be shown. Thus, studies using widely different methods (cellular rodent studies, PET, VBM, fMRI and rsfMRI) all hint at an involvement of the cerebellum in thyroid disease and associated cognitive changes. We thus hypothesize that changes in subclinical hypothyroidism may at least partially be mediated by the posterior cerebellum, connected to the medial frontal cortex.

The medial frontal cortex and the precuneus are involved in the default mode network (DMN) resting state network. This complements the study of Kumar et al. (2018) analyzing alterations in subjects with subclinical hypothyroidism using resting state fMRI. The medial frontal cortex is an important contributor to cognitive control (Ridderinkhof et al., 2004) and seems essential for mediating decision making (Euston et al., 2012). It is possible that the slight memory changes we were able to show in subclinical hypothyroidism may also be mediated by the medial frontal cortex.

4.2. Methodological considerations and limitations of our study

We used a data-driven whole-brain approach without any a priori hypotheses and present our results corrected for multiple comparisons. In contrast, a seed-based analyses would implement a priori seed-regions involving investigator-dependent bias in selection of the regions. Additionally, our approach enables identifying regions not yet documented in the literature. However, the drawback is that we are only able to identify regions with altered whole-brain connectivity. The targets of altered connectivity can only be investigated statistically sound applying a seed-based analysis using an independent data set to avoid so called double dipping (Kriegeskorte et al., 2009). Here, we can only perform a qualitative analysis which allows us to identify regions driving the effect of a decreased degree centrality. Despite that we believe that our approach is helpful in guiding the discussion of the

results and the development of new research hypotheses. The voxel-based degree centrality is a reliable and sensitive marker for changed connectivity. We could show a strong correlation between the degree centrality in full dose levothyroxine and partial withdrawal dose levothyroxine. However, measurements of functional connectivity have a susceptibility to unspecific physiological effects, without being related to levothyroxine dosage intake. We argue that our approach is not prone to non-specific physiological effects, because we analyzed z-transformed degree centrality values and performed regression of nuisance covariates from the data (white matter and CSF signals). One limitation of the present study is a relatively small sample size and the heterogeneity in patients (11 hashimoto, two other causes of hypothyroidism). To increase the validity of our results, we have however chosen a within-subject design in a well-controlled experimental setting of partial levothyroxine dosage withdrawal. We believe the within-subject design to be superior to a cross-sectional study comparing subjects and controls.

5. Conclusion

Even subclinical hypothyroidism due to a reduction in levothyroxine substitution dose under highly controlled experimental conditions induced deficits in working memory tasks and to an activation of brain areas associated with memory and visual processing functions. This suggests that the human brain may be especially sensitive to even short-term thyroid hormone changes and that slight cognitive changes in subclinical hypothyroidism may at least partially be mediated by the cuneus connected to the cerebellum, posterior and the medial frontal cortex.

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Conflict of interest

None declared.

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