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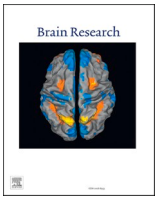

Altered functional connectivity between brain structures in adults with type 1 diabetes and polyneuropathy

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ABSTRACT

Objective: Alterations of the central nervous system are increasingly being recognized as a part of diabetes, especially in the thalamus and the default mode network (DMN). However, the functional involvement in diabetic peripheral neuropathy (DPN) is poorly understood. This study aimed to investigate functional connectivity of thalamus and DMN in individuals with DPN and the associations to clinical characteristics.
Methods: Forty-seven type 1 diabetes mellitus (T1DM) individuals with DPN and 28 healthy controls underwent resting-state functional magnetic resonance imaging. Seed-to-voxel and ROI-to-ROI analyses were performed for thalamus and DMN. The connectivity for both thalamus and DMN were correlated to clinical parameters.
Results: Alterations in the functional connectivity of the thalamus and DMN were observed in individuals with T1DM and DPN. Thalamus showed decreased connectivity to the middle frontal, superior frontal, and precentral cortex (all $p_{\text{FWE-corrected}}<0.05$). DMN ROIs showed increased connectivity to the superior frontal cortex (all $p_{\text{uncorrected}}<0.05$). A trend towards increased overall connectivity within DMN was observed in the T1DM compared to healthy controls ($p=0.051$). The subgroup with painful DPN had significantly increased overall connectivity compared to healthy controls ($p=0.038$). No associations were found to clinical parameters.
Conclusion: Individuals with DPN had disrupted connectivity between thalamus/DMN and other brain structures and disrupted overall mean connectivity within DMN. Our findings support the existing knowledge of central nervous system involvement in diabetes and provide support for the involvement of thalamus and DMN in people with T1DM and DPN.

1. Introduction

Diabetes mellitus is a growing healthcare problem, which according to the International Diabetes Federation affected approximately 463 million adults worldwide in 2019.(Saeedi et al., 2019) One of the most common complications of diabetes is diabetic peripheral neuropathy (DPN), which causes dysfunction of the peripheral nerves.(Russell and Zilliox, 2014) DPN is consistently characterized by loss of protective sensations, typically starting in the toes but slowly progressing proximally to involve the upper limbs in a stocking distribution pattern. (Schreiber, 2015; Juster-Switlyk and Smith, 2016) Up to 50% of all individuals with diabetes develop various degrees of DPN, and approximately 16–26% develop painful DPN, also referred to as neuropathic pain.(Abbott et al., 2011).

Recently, an increasing amount of literature has provided insights into structural and functional changes of the brain in individuals with

Abbreviations: ANOVA, Analysis of variance; BMI, Body mass index; BOLD, Blood-oxygenation level-dependent; DMN, Default mode network; DN4, Douleur Neuropathique 4; DPN, Diabetic peripheral neuropathy; fMRI, Functional magnetic resonance imaging; FWHM, Full-width half-maximum; HbA1c, Hemoglobin A1c; MNI, Montreal Neurological Institute; ROI, Region of interest; SD, Standard deviation; TE, Echo time; TR, Repetition time; T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus; GABA, Gamma(γ)-aminobutyric acid.

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diabetes. A *meta*-analysis of structural brain changes in type 1 diabetes mellitus (T1DM) found reduced gray matter volume.(Moulton et al., 2015) This was in particular evident for the thalamus with bilateral volume loss.(Moulton et al., 2015) Thalamus is a central structure in relaying sensory processing.(Torrico and Munakomi, 2019) Also, recent studies have found lower volume of thalamus in T1DM compared to healthy controls.(Van Duinkerken et al., 2014; Filip et al., 2020) In addition, reduced thalamic volume in T1DM with DPN has been associated with neurochemical dysfunction.(Hansen et al., 2021b) Other studies by Selvarajah and coworkers reported greater relative cerebral blood volume of the thalamus in adults with T1DM and neuropathic pain and increased neuronal activation of the thalamus in T1DM with and without DPN and neuropathic pain during painful heat stimulation. (Selvarajah et al., 2011; Selvarajah et al., 2019) Increased connectivity between thalamus and other areas relevant for sensory processing in adults with T1DM and neuropathic pain has also been shown.(Teh et al., 2021) Taken together, these findings indicate thalamic structural loss and functional dysfunction in T1DM.

Another functional element that has been shown to be altered in diabetes is the default mode network (DMN) (Van Duinkerken et al., 2016; Macpherson et al., 2017; Xia et al., 2018), which is one of the most studied and well-characterized brain networks.(Cui et al., 2015) The DMN is activated at rest and deactivated during tasks or stimulus exposure.(Raichle et al., 2001) Core areas of the DMN include the medial prefrontal cortex, posterior cingulate cortex, and right and left lateral parietal cortices.(Buckner, Andrews-Hanna and Schacter, 2008; Cui et al., 2015) Alterations in the DMN has been shown in cognitive dysfunction (Eyler et al., 2019), normal aging (Damoiseaux et al., 2008; Sala-Llonch, Bartrés-Faz and Junque, 2015), acute and chronic pain (Baliki et al., 2014; Alshelh et al., 2018), but also in conditions like neuropathic pain.(Taskiran Sag et al., 2018). A systematic review of functional brain alterations in people with type 2 diabetes mellitus (T2DM) found reduced connectivity in DMN.(Macpherson et al., 2017) Data in T1DM are, however, conflicting as both altered DMN and no alterations in DMN are reported.(Van Duinkerken et al., 2012; Xia et al., 2018) Moreover, Van Duinkerken and colleagues showed an increase in connectivity between the subgenual cingulate cortex, an area highly involved in mood disorders and the precuneus, a region involved in DMN (Van Duinkerken et al., 2016). Thus, more studies are needed to further explore the alteration of DMN in T1DM.

It is still unknown whether the neuronal alterations in the central nervous system are attributed to diabetes *per se* or secondary to diabetic complications. While some studies have found alterations in T1DM with diabetic complications like microangiopathy, other studies have also found changes in the central nervous system in T1DM without diabetic complications.(Van Duinkerken et al., 2014; Van Duinkerken et al., 2016; Van Duinkerken et al., 2017; Moulton et al., 2015) Additional studies are needed to understand better the underlying neuronal activity changes in T1DM with and without diabetic complications. One way to investigate neuronal activity non-invasively is to use resting-state functional magnetic resonance imaging (fMRI). fMRI provides the possibility to evaluate functional brain-connectivity by detecting spontaneous fluctuations in the blood-oxygenation level-dependent (BOLD) signal reflecting neuronal activity indirectly.(Glover, 2011).

As the thalamus has consistently been reported to be structurally and functionally altered in T1DM, we hypothesized that individuals with T1DM and DPN have altered thalamic functional connectivity compared to healthy controls. Furthermore, we hypothesized that T1DM with DPN reveals alterations in the DMN compared to healthy controls. This study was partly explorative, and the main aims were 1) to investigate the differences in functional connectivity of thalamus and DMN between adults with T1DM and DPN as compared to healthy controls, 2) to explore the clinical associations to these alterations, and 3) to investigate the connectivity parameters in a subgroup of T1DM individuals with neuropathic pain.

2. Results

Table 1 shows the background data for all participants. No group differences were observed in gender (p=0.08) and age (p=0.98), but a higher body mass index (BMI) was present for participants with T1DM compared to healthy controls (p<0.001). One participant with T1DM and painful DPN was not MRI scanned due to claustrophobia. Furthermore, all subjects scanned had head movements equal to or below 1.6 mm (translational) and/or 2.5° (rotation). Hence, all subjects scanned were included in the group-level resting-state fMRI analysis resulting in 28 healthy controls and 47 subjects with T1DM, where 36 T1DM had painless DPN and 11 with painful DPN.

2.1. Thalamus

2.1.1. Seed-to-voxel functional connectivity

Overall T1DM subjects showed decreased functional connectivity between bilateral thalamus and middle frontal-, superior frontal-, and precentral cortex (all $p_{\text{family-wise error(FWE)-corrected}}$<0.011). Increased functional connectivity was demonstrated between the left thalamus and right anterior and posterior insula and between the right thalamus and right occipital cortex (all $p_{\text{uncorrected}}$<0.049). See Table 2 and Fig. 1A. Thalamus seed-to-voxel analysis adjusted for BMI showed almost similar connectivity patterns. See supplementary Table S1.

2.1.2. The associations between the strength of thalamic connectivity and clinical parameters

The z-scores extracted from the region of interest (ROI)-to-ROI analysis, representing the strength of connectivity between the thalamus and right/left middle frontal, superior frontal, and precentral cortex, were used for the correlation analyses to clinical parameters (T1DM duration, HbA1c, glucose level, and composite score). There were no correlations between the mean thalamic connectivity and the clinical characteristics (all p>0.32) (Table 3, Thalamus column).

2.1.3. The association between neuropathic pain and strength of thalamic functional connectivity

A subgroup analysis was performed to explore the possible impact of neuropathic pain on the decreased mean connectivity between the thalamus and left/right middle frontal, superior frontal, and precentral cortex. The diabetes group was divided into participants with and without neuropathic pain and compared to healthy controls. Overall, the mean thalamic functional connectivity strength differed between the groups (p=0.001). The connectivity was lower in T1DM without pain (mean z-score: −0.09 ± 0.18) and T1DM with pain (mean z-score: −0.16 ± 0.21) compared to healthy controls (mean z-score: 0.03 ± 0.20) (Fig. 2). Bonferroni post-hoc analysis revealed that the lower

Table 1
Background data. Data are presented as mean ± SD unless otherwise stated. Abbreviations: BMI, body mass index; T1DM, type 1 diabetes mellitus; HbA1c, hemoglobin A1c.

Characteristics	T1DM (n=48)	Healthy controls (n=28)	*p*-value
Gender (male/female)	38/10	17/11	0.08
Age (years)	50.0 ± 8.5	49.9 ± 11.9	0.98
BMI (kg/m^2)	28.3 ± 4.4	24.6 ± 2.6	<0.001
T1DM duration (years)	32.2 ± 9.5		
Age at onset of diabetes (years)	17.8 ± 8.8		
HbA1c (mmol/mol)	65.8 ± 10.2		
Neuropathy composite score	10.1 ± 4.1		
Actual glucose level (mmol/L)[a]	8.6 ± 3.7		
Diabetic neuropathic pain (*n* (%))	12 (25.0)[b]		

[a] n = 45.
[b] One participant was not magnetic resonance imaging scanned.

Table 2
Thalamus seed-to-voxel functional connectivity. Uncorrected significant threshold at p=0.001 and FWE-corrected threshold at p<0.05 are reported together with cluster size equal to or above 100 voxels (mm^3). Abbreviations: FWE, family-wise error; L, left; MNI, Montreal Neurological Institute; R, right; ROI, region of interest.

Source ROI	Contrast	Regions	Peak MNI coordinates (x, y, z)	Cluster size (mm^3)	*p*-value Uncorrected	*p*-value FWE-corrected
Thalamus, L	T1DM < Healthy	R, Precentral cortex R, Middle frontal cortex	28, −2, 46	254	0.006	–
Thalamus, R		L, Middle frontal cortex L, Superior frontal cortex L, Precentral cortex	−30, 2, 48	412	0.001	0.008
Thalamus, R		R, Middle frontal cortex R, Superior frontal cortex R, Precentral cortex	26, 2, 46	381	0.001	0.011
Thalamus, L	T1DM > Healthy	R, Anterior insula R, Posterior Insula	40, 4, −8	117	0.049	–
Thalamus, R		R, Occipital cortex	14, −98, 22	152	0.026	–

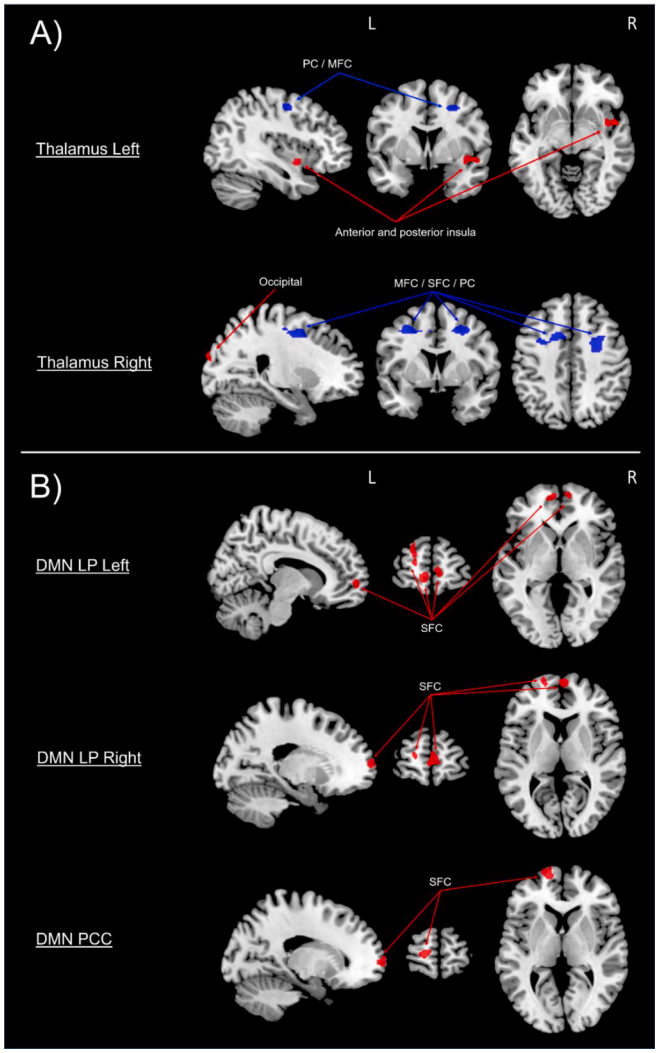

Fig. 1. Seed-to-voxel functional connectivity between A) Thalamus and other brain regions and B) default mode network and other brain areas. Red represents increased connectivity. Blue represents decreased connectivity. Abbreviations: DMN, default mode network; L, left; LP, lateral parietal; MFC, middle frontal cortex; PC, Precentral cortex; PCC, posterior cingulate cortex; R, Right; SFC, superior frontal cortex.

connectivity between T1DM without pain and healthy controls was significant (p=0.041) as well as between T1DM with pain and healthy controls (p=0.017). However, no differences were observed in comparing T1DM with and without pain (p=0.839). The thalamus

Table 3
Thalamus: Correlation between the strength of thalamus to middle frontal, superior frontal, and precentral cortex connectivity and clinical parameters. Default mode network: Correlation between the strength of default mode network connectivity and clinical parameters. DMN, default mode network; HbA1c, hemoglobin A1c; T1DM, type 1 diabetes mellitus.

Clinical parameters	*Thalamus* Correlation coefficient and *p*-value		*Default mode network* Correlation coefficient and *p*-value	
Age	r = -0.073	p = 0.625	r = -0.046	p = 0.760
T1DM duration	r = -0.060	p = 0.690	r = -0.147	p = 0.323
HbA1c	r = -0.120	p = 0.424	r = -0.022	p = 0.883
Actual glucose level[a]	r = -0.115	p = 0.462	r = -0.025	p = 0.875
Composite score	r = -0.134	p = 0.368	r = -0.024	p = 0.873

[a] n = 45.

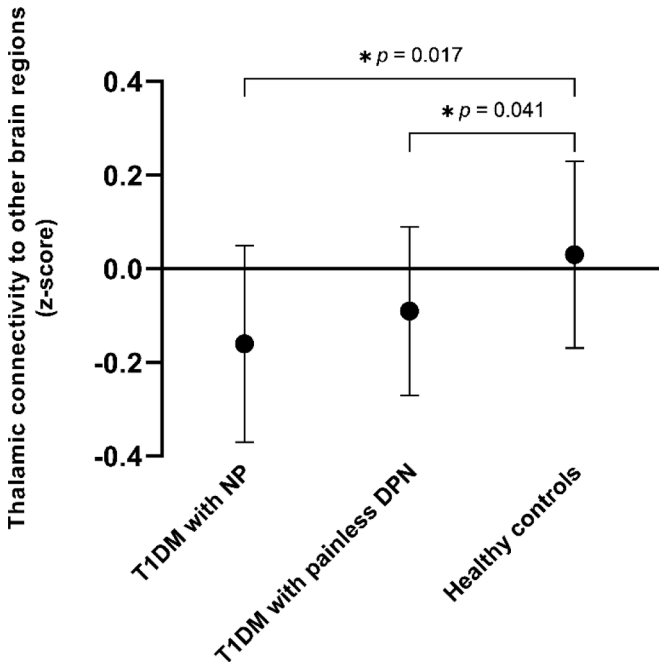

Fig. 2. The mean functional connectivity between the thalamus and left/right middle frontal, superior frontal, and precentral cortex in T1DM with neuropathic pain, T1DM with painless DPN, and healthy controls. *P*-values from Bonferroni post-hoc analysis are reported. Abbreviations: NP, neuropathic pain; T1DM, type 1 diabetes mellitus.

connectivity analysis adjusted for BMI showed almost similar connectivity patterns. See supplementary section 2.

2.2. Default mode network

2.2.1. Seed-to-voxel functional connectivity

Overall, functional connectivity between ROIs in DMN and other brain areas was increased in T1DM compared to healthy controls. Specifically, increased functional connectivity was observed between lateral parietal/posterior cingulate cortex and superior frontal cortex (all $p_{uncorrected} < 0.049$), see Table 4. No significant differences were observed in the connectivity between the medial prefrontal cortex and other voxels in the brain ($p_{uncorrected}>0.05$). See Table 4 and Fig. 1B. DMN seed-to-voxel analysis adjusted for BMI showed almost similar connectivity patterns. See supplementary Table S2.

2.2.2. The strength of overall connectivity within default mode network and its association to clinical parameters

There was a trend towards increased overall connectivity within the DMN for T1DM (mean z-score: 0.58 ± 0.18) compared to healthy controls (mean z-score: 0.50 ± 0.17) (p=0.051), see Fig. 3A. No correlations were found between overall connectivity within DMN and clinical parameters (T1DM duration, HbA1c, glucose level, and composite score) (all $p>0.32$). Data are presented in Table 3, Default mode network column. The DMN connectivity analysis adjusted for BMI showed almost similar connectivity patterns. See supplementary section 4.

2.2.3. The association between neuropathic pain and strength of functional connectivity of DMN

A subgroup analysis was performed to explore the possible impact of neuropathic pain on the functional connectivity within the DMN in the subgroups T1DM without pain (mean z-score: 0.56 ± 0.18), T1DM with neuropathic pain (mean z-score: 0.66 ± 0.18), and the healthy control group (mean z-score: 0.50 ± 0.17). The within connectivity in DMN was significantly different between the groups (p=0.041). Bonferroni post-hoc analysis showed increased DMN connectivity in T1DM with neuropathic pain compared to the healthy control group (p=0.038), but no other group differences were significant (see Fig. 3B). The subgroup DMN connectivity analysis adjusted for BMI showed almost similar connectivity patterns. See supplementary section 5.

Table 4
Default mode network seed-to-voxel functional connectivity. All $p_{FWE\text{-}corrected}>0.05$, hence uncorrected significant threshold at p=0.001 are reported together with cluster size equal to or above 100 voxels (mm^3). Abbreviations: DMN, default mode network; FWE, family-wise error; L, left; LP, lateral parietal; MNI, Montreal Neurological Institute; PCC, posterior cingulate cortex; R, right; ROI, region of interest.

Source ROI	Contrast	Regions	Peak MNI coordinates (x, y, z)	Cluster size (mm^3)	p-value Uncorrected
DMN LP, L	T1DM > Healthy	Superior frontal cortex, L, R	−8, 60, −6	189	0.014
DMN LP, L		Superior frontal cortex, L, R	−20, 62, 26	116	0.045
DMN LP, R		Superior frontal cortex, L, R	−18, 64, 10	112	0.049
DMN LP, R		Superior frontal cortex, L, R	4, 64, 4	175	0.017
DMN PCC		Superior frontal cortex, L	−18, 68, 4	148	0.026

3. Discussion

In this resting-state fMRI study, individuals with type 1 diabetes and peripheral neuropathy showed decreased thalamic connectivity to both left and right middle frontal, superior frontal, and precentral cortex compared to healthy controls. The strength of this functional connectivity was even lower in individuals with neuropathic pain than those with painless diabetic peripheral neuropathy. The overall connectivity within the default mode network was increased, and these findings were more pronounced in patients with painful neuropathy than those without pain. Default mode network showed increased connectivity between the lateral parietal/precentral cortex and the superior frontal cortex in people with type 1 diabetes and peripheral neuropathy.

The findings confirm that functional changes in the brain may accompany individuals with type 1 diabetes and peripheral neuropathy.

3.1. Thalamic connectivity in diabetes

Our study supports the previous findings indicating thalamic dysfunction in T1DM with DPN. Several studies have suggested that structural brain changes might be associated with functional brain responses.(Schmidt et al., 2014; Gao et al., 2018) The gray matter volume of the thalamus in the current study cohort was investigated by Hansen and coworkers. Lower bilateral thalamic volume was found in the T1DM group compared to healthy controls.(Hansen et al., 2021a) This is a possible reason for the alteration of the functional connectivity pattern of thalamus in the T1DM group of this study. We found decreased connectivity strength between the thalamus and the middle frontal, superior frontal, and precentral cortex. Also, volume loss of the right precentral cortex and reduced cortical thickness of several frontal gyri, including the superior frontal cortex, were found in the current cohort. (Hansen et al., 2021a) The precentral cortex locates the primary motor cortex (Banker and Tadi, 2021) and is also a major component in the sensorimotor brain network, which subserves motor and sensory tasks. (Damoiseaux et al., 2006) Van Duinkerken et al. found increased connectivity in the precentral cortex in adults with T1DM without any neuropathic complications compared to a control group.(Van Duinkerken et al., 2012).

Moreover, the lack of differences in connectivity findings between patients with and without painful DPN suggests that alterations in the thalamic connectivity patterns are not limited to one of the subgroups. However, a recent study by Selvarajah and coworkers investigated the thalamic connectivity to other brain regions and greater connectivity to the insula and the somatosensory cortex in T1DM with irritable painful DPN compared to non-irritable painful DPN was reported.(Teh et al., 2021) Since the study did not include healthy controls, it was not possible to conclude whether the alterations are specific for neuropathic pain compared to a healthy control group.(Teh et al., 2021) The insula is recognized as a key structure involved in pain processing, and in particular, the posterior insula is involved in the sensory perception and processing of pain.(Petrou et al., 2012) Greater thalamic-insula connectivity was also observed in our study in the T1DM group. However, the results did not survive the correction of multiple comparisons and should be interpreted with caution.

Furthermore, the strength of thalamic connectivity was not associated with clinical and demographic parameters, including age, T1DM duration, HbA1c, glucose level, and neurophysiological composite score, suggesting that these factors do not contribute to the connectivity changes reported in the current study.

3.2. Default mode network connectivity in diabetes

In comparison to healthy controls, persons with T1DM demonstrated a trend toward increased overall connectivity within DMN, and a significant increase in the connectivity was shown in T1DM with neuropathic pain. This is supported by other studies reporting association

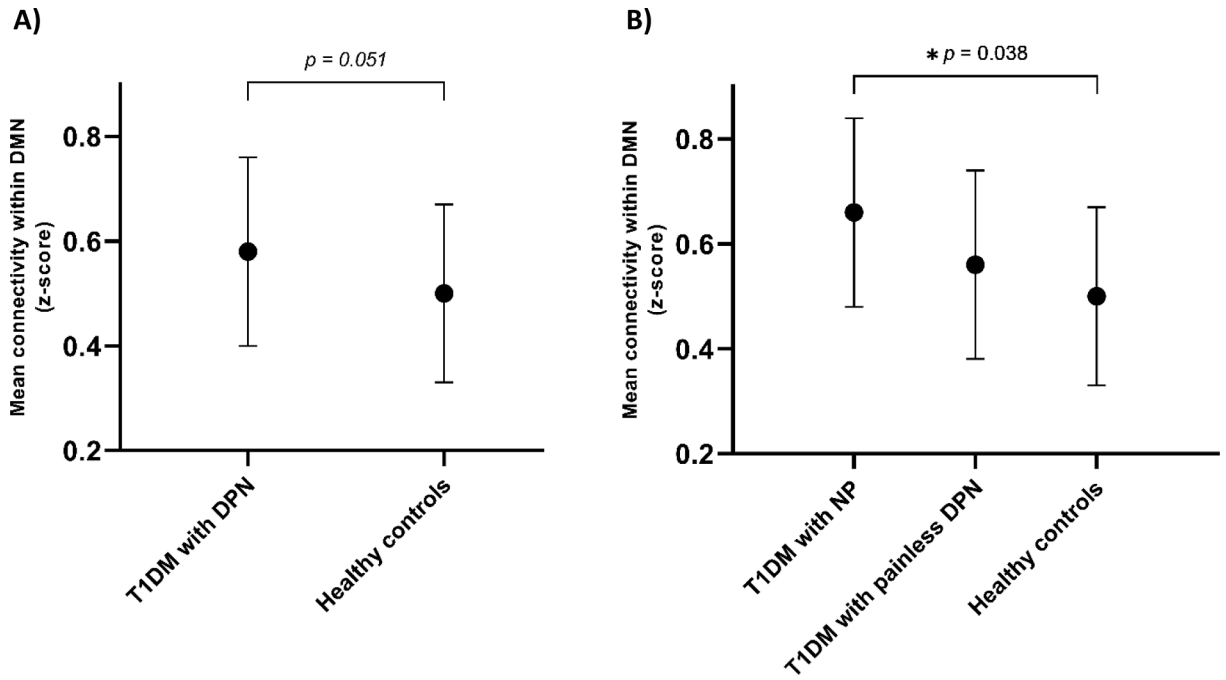

Fig. 3. Functional connectivity within default mode network in A) T1DM with DPN and healthy controls and B) T1DM with neuropathic pain, T1DM with painless DPN, and healthy controls. For Fig. 3B, *p*-values from Bonferroni post-hoc analysis are reported. Abbreviations: DMN, default mode network; NP, neuropathic pain; T1DM, type 1 diabetes mellitus.

between pain and increased connectivity to the posterior cingulate cortex, which is one of the regions involved in DMN (Nielsen, Balslev and Hansen, 2005), and in particular, association between painful neuropathy and increased connectivity in DMN.(Taskiran Sag et al., 2018) However, with a small sample size of the subgroup experiencing mild neuropathic pain, these results should be investigated further in more extensive studies. Other studies have reported conflicting results regarding DMN connectivity. Van Duinkerken et al. reported no changes in the DMN in T1DM with and without macroangiopathic complications, while Xia et al demonstrated increased spontaneous activity of DMN in T1DM.(Van Duinkerken et al., 2012; Xia et al., 2018) However, the latter included T1DM without any complications.

Even though no clusters in the current study survived the FWE-corrected threshold in the ROI-to-ROI analysis of DMN, uncorrected results were consistently found as increased connectivity between DMN ROIs and the superior frontal cortex. These findings in T1DM are consistent with those in T2DM, where it is demonstrated increased connectivity in DMN primarily involving bilateral superior frontal gyrus.(Cui et al., 2015) However, their cohort consisted of mixed phenotypes, including individuals with/without DPN making it difficult to interpret whether the underlying connectivity changes were attributable to DPN. On the other hand, in the same cohort as our patients, Nissen et al. showed prolonged conduction time in central pathways with electrophysiological studies.(Nissen et al., 2020) This reflects changes in nerve integrity, synaptic transmission, and cortical processing, indicating extensive alterations of the central response and supporting the current study's findings.(Nissen et al., 2020) Increased connectivity between DMN and the superior frontal cortex has previously been reported in patients with mild cognitive impairment, especially in the memory domain.(Li et al., 2017) Furthermore, several studies have linked DPN to the presence of cognitive impairment in diabetes, including T1DM.(Ryan et al., 1992; Brands et al., 2005; Nunley et al., 2015; Ding et al., 2019) Therefore, one might speculate if the increased connectivity between DMN and the superior frontal cortex could indicate changes in cognitive function. However, this study did not include cognitive data. Hence such a hypothesis was not possible to either confirm or reject.

Our results showed no association between the strength of overall connectivity within the DMN and age, duration of T1DM, HbA1c, glucose levels, or the neurophysiological composite score. This is in accordance with earlier observations in both T1DM and T2DM, where no associations between DMN connectivity and clinical parameters were reported (Cui et al., 2015; Xia et al., 2018). However, the same study found significant associations between DMN connectivity to cognitive tasks, which additionally verify, that these networks seem to be disrupted in parallel with cognitive decline in T1DM. Finally, a study demonstrated that DMN connectivity significantly correlated with diabetes duration in T2DM, but the cohort was not characterized with DPN. (Liu et al., 2019) Hitherto, only limited and ambiguous results are presented in the literature, and hence further research should be undertaken to investigate the clinical and cognitive relevance of DMN changes in T1DM.

3.3. Limitations

The results of this study have several limitations. We included only T1DM with verified neuropathy. This limits the external validity, as we do not know whether the observed changes are restricted to diabetes *per se* or the presence of DPN. Assessment of neuropathic pain was concluded based on the 7-items self-reported DN4 questionnaire, and in the current cohort, the neuropathic pain subgroup was relatively small, limiting the statistical power. Investigations of a larger cohort, including thoroughly well-characterized phenotypes, are needed to confirm our results. Also, the use of centrally acting medication was not a part of the exclusion criteria, which could potentially have influenced the resting-state fMRI results. Factors like age and BMI might also have an impact on brain function.(Duncan and Northoff, 2013) However, the participants were matched on age; hence this factor was partly eliminated. Moreover, additional analyses were performed to correct for BMI, and similar patterns of connectivity differences were observed.

This study extracted the DMN network using the four predefined DMN ROIs from CONN-toolbox.(Whitfield-Gabrieli and Nieto-Castanon, 2012) Hence, the network might not be specific for the individuals in this study. However, the CONN-toolbox utilizes an independent-

component-analyses (ICA) of 497 subjects to create these ROIs. This high number of participants included makes this method standardized. However, it is noteworthy that these methodological considerations should be taken into account when interpreting the results. Furthermore, the predefined thalamus structure could be further investigated in subregions relevant to the sensory processing of pain. Since this study was partly explorative, we also included the results of not surviving the FWE-corrected threshold. These results must be interpreted with caution.

4. Conclusion

Our study suggests alterations of thalamic and default mode network functional connectivity in individuals with T1DM with confirmed peripheral neuropathy compared to healthy controls. We demonstrated a disrupted pattern in the functional connectivity for the thalamus, but no associations were shown to clinical parameters. Increased connectivity within the default mode network was most pronounced in patients with painful DPN, reflecting a functional impact of mild neuropathic pain. Our findings support the existing knowledge of central nervous system involvement in diabetes and provide support for the involvement of thalamus and default mode networks in diabetic peripheral neuropathy. Further studies should include a group of T1DM without DPN, a T1DM group with painless DPN, and a group with painful DPN to deeply investigate functional alterations in the different phenotypes.

5. Experimental procedures

5.1. Participants

This study is based on secondary analyses from a larger clinical trial conducted at Aalborg University Hospital, Denmark.(Brock et al., 2019) From the Department of Endocrinology, Aalborg University Hospital, the study included 48 subjects with a verified diagnosis of T1DM for minimum two years (hemoglobin A1c (HbA1c) $\geq$ 7%) and confirmed DPN, based on the Toronto criteria.(Tesfaye et al., 2010). Individuals were pre-screened based on vibration threshold equal to or above 18 V, and the diagnose was ensured by abnormal neurophysiological testing. Furthermore, 28 healthy controls were included. Adults with age equal to or above 18 years were included. The exclusion criteria were, among others, T2DM, other neurological and/or psychiatric diseases, malignant neoplasms requiring chemotherapy, surgery, radiation, or palliative care in the previous 5 years, known abuse of alcohol and/or medicine, pregnancy, and body mass index (BMI) lower or equal to 20 kg/m^2. All participants provided informed written consent before enrollment in the trial. Ethical approvals were granted by The North Denmark Region Committee on Health Research Ethics (N-20130077, N-20090008) and registered with clinicaltrials.gov (ref NCT02138045).

5.2. Clinical parameters

Information on the demographical and clinical characteristics, including gender, age, and diabetes duration, were obtained, and HbA1c was analyzed in blood samples at the Department of Clinical Biochemistry, Aalborg University Hospital. Morning blood glucose levels were measured on the scan day. Seven-items "Douleur Neuropathique 4" (DN4) questionnaire was fulfilled where a score equal to or above 3 indicates neuropathic pain.(Bouhassira et al., 2005; Spallone et al., 2012) The peripheral nerve conduction study included measurement of: sensory nerve action potential amplitudes of the sural nerve, motor nerve conduction velocities and compound muscle action potential amplitudes of the common peroneal nerve, and motor nerve distal latencies for both common peroneal and tibial nerves. To evaluate the severity of DPN, these five components were used to calculate an overall composite score (range 0–15) derived from transformed percentile points of abnormalities in nerve conduction studies. A total score of 3 points indicates DPN.(Dyck et al., 1997; Brock et al., 2019).

5.3. Image acquisition

MRI was collected using a 3 T GE scanner (GE Signa HDxt, General Electric, Milwaukee, WI, USA). An eight-channel head coil was used, and the head was fixed using foam pads. For anatomical registration of the fMRI scans, a high-resolution T1-weighted three-dimensional structural scan (BRAVO) was obtained before the resting-state fMRI scan with the following parameters: Repetition time (TR) 9.0 ms, echo time (TE) 3.6 ms, flip angle 14°, matrix 320×320, field of view 25 cm, and voxel size 0.8×0.8×1.0 mm. The participants were instructed to relax, close their eyes, but not sleep, and try to empty their brains by not thinking of anything specific during the resting-state fMRI acquisition. For the resting-state fMRI, 192 volumes of gradient echo-planar images were acquired with: TR 2000 ms, TE 30 ms, flip angle 90°, matrix 64x64, field of view 24 cm, voxel size 2.5×2.5×3.8 mm, and scan time 6 min and 24 s.

5.4. Functional MRI preprocessing

Prior to the preprocessing of the resting-state fMRI data, the images were visually inspected for artifacts. The preprocessing step was executed in CONN toolbox version 18b (https://www.nitrc.org/projects/conn) (Whitfield-Gabrieli and Nieto-Castanon, 2012) running in MATLAB (version 9.7.0.1319299 *(R2019b)*, Natick, Massachusetts: The MathWorks Inc.) The default preprocessing pipeline in CONN toolbox was used, including the following steps: motion correction, slice timing correction, ART-based outlier detection, segmentation of cerebrospinal fluid, white matter and gray matter, and normalization to standard Montreal Neurological Institute (MNI) brain template for both structural and functional images, and smoothing with an 8 mm full-width half-maximum (FWHM) Gaussian kernel. The voxel size after normalizing was 2.0×2.0×2.0 mm. During denoising, the signal from white matter, cerebrospinal fluid, and the six head motion parameters derived from spatial motion correction were added as a confounder (aCompCor strategy implemented in the CONN toolbox to control for physiological and movement confounders).(Patriat, Molloy and Birn, 2015; Zhu et al., 2017; Pelletier-Baldelli, Andrews-Hanna and Mittal, 2018) Data were band-pass filtered to a frequency window of 0.008–0.09 Hz.(ref).

5.5. Functional connectivity with seed-to-voxel analyses

To examine the difference in resting-state fMRI connectivity between the T1DM group and healthy controls, seed-to-voxel correlation analyses were performed for ROIs (also named seeds in CONN toolbox). This analysis characterizes the connectivity pattern between predefined ROIs and the rest of the brain. The following predefined ROIs from CONN toolbox were used in the seed-to-voxel analyses; thalamus (bilateral) and DMN ROIs (medial prefrontal cortex, left and right lateral parietal cortex, and posterior cingulate cortex). The ROIs used covered the whole structure of the chosen area. For instance, the connectivity was investigated between the entire thalamus and voxels in the rest of the brain. The average of the time-course signal from the voxels in each ROI was correlated with the time course of each voxel in the entire brain using Pearson's correlation coefficients to obtain functional connectivity maps. SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK) was used for the seed-to-voxel group-level analyses.(Brett et al., 2002) Two sample t-tests were used to compare the seed-to-voxel connectivity between the groups. Data adjusted for BMI were also analyzed. The primary significance thresholds were set to $p \leq 0.001$ and presented with clusters equal to or above 100 voxels (mm^3). Finally, cluster-level statistics were presented with both $p < 0.05$, FWE corrected, and $p < 0.05$, uncorrected.

5.6. Functional connectivity with ROI-to-ROI analyses

In contrast to seed-to-voxel analysis, which characterizes the pattern between a predefined ROI and the rest of the brain, an ROI-to-ROI analysis was performed to characterize the connectivity pattern between two predefined ROIs. ROI-to-ROI analyses were performed for both the thalamus and DMN. For the thalamus, the ROI-to-ROI analyses with Fisher's r-to-z transformation were applied to examine the strength of the functional connectivity between the thalamus ROI and regions, demonstrating an FWE-corrected *p*-value lower than 0.05 in the seed-to-voxel analysis. The ROIs for these analyses were extracted from MarsBar 0.44 toolbox.(Brett, 2016) ROI-to-ROI analyses with Fisher's r-to-z transformation were also applied between each DMN ROI and to every other DMN ROIs in each subject. The average z-scores referred to as the overall connectivity within DMN were used as the strength of DMN connectivity and compared between diabetes and healthy control groups. The output of the ROI-to-ROI analyses was used for further subgroup analyses and correlation analyses.

5.7. Statistical analyses

Data are presented as mean ± standard deviation (SD) unless otherwise stated. The assumption of normal distribution was checked using Q-Q plots. Two sample t-tests were used to determine any group difference in age and BMI and differences in functional connectivity in seed-to-voxel- and ROI-to-ROI analyses of thalamus and DMN. A chi-squared test was used for gender analysis. Using analysis of variance (ANOVA), subgroup analyses were performed to explore the possible impact of neuropathic pain on the strength of functional connectivity of the thalamus and within DMN. Analysis of covariance (ANCOVA) was applied to analyze data adjusted for BMI. Bonferroni post-hoc test was used to correct multiple comparisons in the subgroup analyses. Using Pearson correlation's tests, ROI-to-ROI data were also correlated to clinical parameters (T1DM duration, HbA1c, glucose level, and composite score). Analyses were performed in IBM SPSS Statistics (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). $P<0.05$ was considered significant.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brainres.2022.147882.

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