Magnetic Resonance Neuroimaging Study of Brain Structural Differences in Diabetic Peripheral Neuropathy

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OBJECTIVE

Diabetic peripheral neuropathy (DPN) has hitherto been considered a disease of the peripheral nervous system only, with central nervous system (CNS) involvement largely overlooked. The aim of this study was to investigate any differences in brain structure in subjects with DPN.

RESEARCH DESIGN AND METHODS

Thirty-six subjects with type 1 diabetes (No DPN [n = 18], Painful DPN [n = 9], Painless DPN [n = 9]) underwent neurophysiological assessment to quantify the severity of DPN. All subjects, including 18 healthy volunteers (HVs), underwent volumetric brain magnetic resonance imaging at 3 Tesla.

RESULTS

Adjusted peripheral gray matter volume was statistically significantly lower in subjects with painless and painful DPN (mean 599.6 mL [SEM 9.8 mL] and 585.4 mL [10.0 mL], respectively) compared with those with No DPN (626.5 mL [5.7 mL]) and HVs (639.9 mL [7.2 mL]; ANCOVA, P = 0.001). The difference in adjusted peripheral gray matter volume between subjects with No DPN and HVs, and those with Painful DPN and Painless DPN was not statistically significant (P = 0.16 and 0.30, respectively). Voxel-based morphometry analyses revealed greater localized volume loss in the primary somatosensory cortex, supramarginal gyrus, and cingulate cortex (corrected P < 0.05) in DPN subjects.

CONCLUSIONS

This is the first study to focus on structural changes in the brain associated with DPN. Our findings suggest increased peripheral gray matter volume loss, localized to regions involved with somatosensory perception in subjects with DPN. This may have important implications for the long-term prognosis of DPN.

Diabetic peripheral neuropathy (DPN) is a common, debilitating, and distressing complication that develops in up to 30–50% of patients with diabetes (1). A painless distal symmetrical sensorimotor neuropathy, which increases the risk of foot ulceration and subsequent amputation, develops in most patients. In a significant proportion of patients, a chronic painful condition also develops, which can result in considerable disability and suffering. Although various vascular and metabolic factors (2) have been implicated, a complete understanding of the pathogenesis of DPN remains elusive (3,4). DPN has hitherto been considered a disease of the peripheral
nervous system only, with central nervous system (CNS) involvement largely overlooked. Although some evidence for CNS involvement has recently emerged (5), further knowledge of the extent of this involvement, which is now possible with advances in noninvasive magnetic resonance imaging (MRI) (6), is crucial for a greater understanding of the pathologic mechanisms of DPN.

We have previously demonstrated a clear reduction in cross-sectional area of the spinal cord in patients with established DPN (7). More recently, we reported that this reduction in spinal cord volume was found early in the neuropathic process in DPN, even in patients with subclinical DPN (8). The fact that this appears to occur early in the neuropathic process in DPN is worrying as this may indicate irreversible damage. Recently, there have been further reports of CNS involvement in DPN including dysfunction of the somatosensory afferent pathways using evoked potentials, thalamic neuronal dysfunction, and perfusion abnormalities seen on MRI studies (9–11). These findings point to a significant involvement of the CNS in DPN, leading us to investigate whether there are structural changes in the brain accompanying these functional abnormalities.

Although the cerebral complication of diabetes is well-recognized, there are no previous reports of structural changes in the brain that are specifically associated with DPN (12,13). Previous neuroimaging studies have focused on either acute cerebral disturbances of diabetes (14) in the context of metabolic abnormalities (e.g., hypoglycemia, ketoacidosis) (15) and stroke (16), or the long-term effects of diabetes on brain atrophy and cognition in the context of diabetes-related conditions (e.g., obesity, hyperinsulinemia) and dementia (17,18). While these metabolic and vascular abnormalities impact the brain as a whole, the aim of our study was to investigate differences in brain structure in subjects with DPN, a predominantly distal sensory motor neuropathy. In particular, we examined for evidence of structural brain changes in subjects with DPN compared with age-matched healthy volunteers (HVs) and patients without DPN. Because of the cross-sectional nature of this study, we could not examine the precise temporal or causal nature of any changes related to plasticity.

**RESEARCH DESIGN AND METHODS**

We performed a cross-sectional, observational, case-control cohort study. Patients with type 1 diabetes attending Sheffield Teaching Hospitals NHS Foundation Trust outpatient service were screened for the study between January 2009 and March 2012. To be eligible, subjects had to meet the following inclusion criteria: type 1 diabetes diagnosed for >5 years; right-handed; age between 18 and 65 years; and stable glycemic control (HbA1c <11% [97 mmol/mol]). We excluded patients for the following reasons: clinical evidence of disease in the CNS (e.g., cerebrovascular disease); presence of nondiabetic neuropathies; history of alcohol consumption of >20 units/week (1 unit is equivalent to 1 glass of wine or 1 measure of spirits); presence of diabetic neuropathies other than DPN (e.g., mononeuropathies or proximal motor neuropathies); presence of epilepsy; recurrent severe hypoglycemia; hypoglycemic unawareness; and psychiatric conditions, claustrophobia, or other factors that preclude MRI. We also recruited age-matched and sex-matched, right-handed, nondiabetic HVs. All control subjects were free of chronic pain conditions and were not using analgesic medications or alternative therapies for treatment of pain. All subjects gave written informed consent before participating in the study, which had prior approval by the Sheffield Local Research Ethics Committee (reference no. 08/H1308/276).

**DPN Assessments**

Neuropathy symptoms were documented by completion of a standard Neuropathy Symptom Score questionnaire. Then the outcome of a detailed neurological examination was graded by defined criteria according to the standard Neuropathy Impairment Score questionnaire (19).

All subjects also underwent the following neurophysiological assessments: 1) quantitative sensory assessments (vibration and cooling detection thresholds) were acquired from the dorsal aspect of the right foot using the Computer Assisted Computer Assisted Sensory Evaluation IV (W.R. Electronics, Stillwater, MN) system using standard techniques (20,21); 2) cardiac autonomic function tests performed with a computer-assisted technique according to O’Brien’s protocol (22); and 3) nerve conduction studies performed at a stable skin temperature of 31°C and a room temperature of 24°C using a Medelec electrophysiological system (Synergy Oxford Instruments, Oxford, U.K.). The following nerve attributes were measured: 1) sural sensory nerve action potentials and conduction velocities; and 2) common peroneal and tibial motor nerve distal latency, compound muscle action potential, and conduction velocity. An overall neuropathy composite score (NCS) derived from transformed percentile points of abnormalities in nerve conduction studies, vibration detection thresholds, and heart rate variability with deep breathing was calculated. On the basis of these clinical and neurophysiological assessments, subjects with diabetes were divided into the following three groups according to severity of neuropathy based on the Toronto Consensus statement (23): 1) No DPN group, consisting of asymptomatic subjects with normal clinical and neurophysiological assessment findings; 2) Painless DPN group, consisting of subjects with painless neuropathy, abnormal clinical findings, and at least two abnormalities found on neurophysiological assessments; and 3) Painful DPN group, comprising subjects with chronic painful symptoms for a duration of at least 6 months with both clinical and neurophysiological abnormalities.

**Diabetic Retinopathy Grading**

It has been suggested that hyperglycemia-associated cerebrovascular disease may underlie structural reductions in cerebral gray and white matter volume in type 1 diabetes (24). To account for this in our study, we used diabetic retinopathy (DR) not only as a marker of chronic hyperglycemia but also as a “window” to the brain (25). As an extension of the CNS, the retina and retinal vessels display similarities to the brain in terms of embryological origins, anatomy, physiological characteristics, and response to insult (e.g., the metabolic insult of diabetes) (26,27). Hence, abnormalities of the retinal arterioles (e.g., microaneurysms, retinal hemorrhages, and arteriolar narrowing) may be markers of concomitant cerebral small-vessel disease (27). We graded DR severity (0, no DR; 1, mild nonproliferative DR; 2, moderate/severe nonproliferative and proliferative DR) using...
images (two-field mydriatic digital photography) acquired as part of the National Diabetic Retinopathy Screening Program.

**Brain Imaging**

High resolution, three dimensional T1-weighted MRI scans were acquired using a magnetization-prepared rapid gradient echo sequence at 3 Tesla (Achieva, Philips Healthcare, Best, The Netherlands). Scanning parameters were as follows: flip angle = 80; echo time (TE) = 3.1 ms; repeat time (TR) = 6.9 ms; inversion time (TI) = 8.2 s; reconstructed voxel size = 0.83 × 0.83 × 0.90 mm³ and sequence scan time = 12 min.

**Statistical Analysis**

Baseline characteristics were described as the mean (SD) for normally distributed variables, and as the median (interquartile range) for variables with a skewed distribution (using the statistical package SPSS version 20.0). All structural data were analyzed using FSL-SIENAX and FSL-VBM (www.fmrib.ox.ac.uk/fsl) protocols carried out with FSL tools using default settings. The analysis of structural data was conducted in a two-step process.

**Step 1: Brain Tissue Volume Quantification and Analysis**

Brain tissue volumes (total gray matter, white matter, peripheral gray matter, and ventricular cerebrospinal fluid [CSF]) normalized for subject head size were estimated for each subject using FSL-SIENAX (28). Briefly, brain and skull images for each subject were extracted from the single whole-head MRI structural images. The brain image was then affine-registered to Montreal Neurological Institute 152 space (29,30) (using the skull image to determine the registration scaling); this is primarily performed in order to obtain the volumetric scaling factor, to be used for normalization of head size. Next, tissue-type segmentation with partial volume estimation is carried out (31) in order to calculate volumes of each brain tissues types.

We used a univariate test (ANCOVA) to compare differences among groups (HV, No-DPN, Painful-DPN, and Painless-DPN) by calculating mean volumes for each brain tissue type per group adjusted for age and severity of DR, with sex and group as fixed factors. A full factorial model was used, with group difference as a contrast. Hence, only changes attributed to DPN neuropathy were reported. The relation between mean brain volumes and individual attributes of nerve function (e.g., nerve conduction velocities, vibration detection threshold, and others) and NCS was analyzed in more detail among subjects with diabetes (n = 36) using Spearman correlation coefficients.

**Step 2: Brain Morphology Quantification and Analysis**

Next, we performed a more detailed analysis to quantify the neuroplastic changes that occur in association with DPN. This was investigated using the widely applied model for assessing brain morphology by quantifying and comparing the relative concentrations of gray matter throughout the brain between two groups. This analysis is performed using a voxel-by-voxel-based method, termed voxel-based morphometry (VBM). Briefly, we used FSL-VBM, which is an optimized VBM protocol (32) carried out with FSL tools (33). First, structural images were brain-extracted, gray matter–segmented, and registered using the same methods described above. The resulting images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific gray matter template. Second, all native gray matter images were nonlinearily registered to this study-specific template and “modulated” to correct for local expansion (or contraction) because of the nonlinear component of the spatial transformation. The modulated gray matter images were then smoothed with an isotropic Gaussian kernel with a σ value of 3 mm. Finally, a voxelwise general linear model was applied using permutation-based nonparametric testing, correcting for multiple comparisons across space (34). P values < 0.05, fully corrected for multiple comparisons, were considered significant.

Alternatively, and where stated, results were uncorrected for multiple comparisons, but we used the accepted more stringent higher threshold of P < 0.001. Using this methodology, we began by comparing brain morphology in patients with DPN (painful and painless combined) versus age-matched and sex-matched HVs and No-DPN patients. Subsequently, we compared the differences in brain morphology between Painful-DPN and Painless-DPN.

**RESULTS**

Table 1 show demographic details and results of the neurophysiological and MRI assessments for the three groups with diabetes (No DPN, n = 18; Painful DPN, n = 9; and Painless DPN, n = 9) and nondiabetic control subjects (n = 18). Subjects were matched for age (ANOVA, P = 0.69), duration of diabetes (P = 0.93), and HbA1c level (P = 0.33). There was no significant difference in NCS between the Painful DPN and Painless DPN groups (P = 0.86). A greater proportion of DPN subjects had more advanced DR (P < 0.001; Table 1). Subjects with advanced DR (preproliferative and proliferative DR, 611.8 mL [4.6 mL]) had lower peripheral gray matter volume compared with those with no DR (625.0 mL [4.6 mL]) and background DR (620.6 mL [2.3 mL]; P = 0.64). Examination of the interaction effect of sex revealed that it did not have a relationship to peripheral gray matter volume that was distinct from the effects of neuropathy or DR status.

**Global Brain Measures**

Figure 1 displays the box-and-whisker plots of peripheral gray matter volume for each of the study cohorts. Based on the mean peripheral gray matter volume adjusted for DR status and age, subjects in both the Painless DPN and Painful DPN groups had lower peripheral gray matter volume (mean [SEM] 599.6 mL [9.8 mL] and 585.4 mL [10.0 mL], respectively) compared with subjects in the No DPN group (626.5 mL [5.7 mL] and HV group (639.9 mL [7.2 mL]). Pairwise comparison revealed that subjects in both the Painful DPN and Painless DPN groups had significantly lower adjusted peripheral gray matter volume compared with subjects in the No-DPN group (P = 0.001, 95% CI −64.4 to −17.8; and P = 0.02, 95% CI −49.5 to −4.2, respectively; ANCOVA, P < 0.0001) and HV group (P < 0.0001, 95% CI −83.3 to −25.7; and P = 0.002, 95% CI −65.3 to −15.1, respectively).

Pairwise comparison of the difference in adjusted peripheral gray matter volume between the No-DPN and HV groups was not statistically significant (P = 0.16, 95% CI −32.1 to 5.3). The difference between the Painful DPN and Painless DPN groups was not statistically significant (P = 0.30, 95% CI −41.8 to 13.3). There was no statistically
significant difference in deep gray matter volume across the four study subgroups (ANCOVA, \(P = 0.22\); Table 1). Total gray matter volume (i.e., combined peripheral and deep gray matter volumes) was statistically significantly lower in subjects with established DPN (i.e., Painful DPN and Painless DPN groups) compared with subjects in the HV and No-DPN groups (ANCOVA, \(P < 0.0001\)). However, this most likely represents a greater reduction in peripheral gray matter volume,

**Table 1—Demographics characteristics, neurophysiological assessments, and brain volumes of study subjects**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HV (n = 18)</th>
<th>No DPN (n = 18)</th>
<th>Painless DPN (n = 9)</th>
<th>Painful DPN (n = 9)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, (n)</td>
<td>8</td>
<td>10</td>
<td>7</td>
<td>5</td>
<td>0.44</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.9 (14.3)</td>
<td>43.0 (10.1)</td>
<td>46.3 (12.1)</td>
<td>46.6 (11.4)</td>
<td>0.69</td>
</tr>
<tr>
<td>HbA(_1c), % [mmol/mol]</td>
<td>8.5 [69.7] (11.8)</td>
<td>8.4 [68.0] (7.6)</td>
<td>9.2 [77.5] (19.6)</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>22.2 (12.7)</td>
<td>22.5 (8.5)</td>
<td>24.5 (11.4)</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>No DR, (n)</td>
<td>3 (16.7)</td>
<td>2 (22.2)</td>
<td>1 (11.1)</td>
<td>(&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>Background DR, (n) (%)</td>
<td>13 (72.2)</td>
<td>3 (33.3)</td>
<td>2 (22.2)</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Preproliferative DR, (n) (%)</td>
<td>1 (5.5)</td>
<td>1 (11.1)</td>
<td>0 (0.0)</td>
<td>0.0004</td>
<td></td>
</tr>
<tr>
<td>Proliferative DR, (n) (%)</td>
<td>1 (5.5)</td>
<td>3 (33.3)</td>
<td>6 (66.6)</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Sural velocity (m/s)</td>
<td>39.4 (5.2)</td>
<td>17.9 (18.4)</td>
<td>11.9 (18.9)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Sural amplitude (mV)</td>
<td>11.4 (6.6)</td>
<td>4.3 (4.6)</td>
<td>2.0 (3.1)</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Peroneal velocity (m/s)</td>
<td>42.2 (4.3)</td>
<td>24.9 (20.8)</td>
<td>21.6 (24.4)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Peroneal amplitude (mV)</td>
<td>1.7 (0.9)</td>
<td>0.9 (0.9)</td>
<td>2.1 (2.6)</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Vibration JND</td>
<td>15.5 (1.9)</td>
<td>18.5 (4.3)</td>
<td>19.9 (3.7)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>NCS</td>
<td>1.9 (1.3)</td>
<td>10.3 (7.3)</td>
<td>9.6 (6.6)</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Peripheral gray matter volume (mL)</td>
<td>639.9 (7.1)</td>
<td>626.5 (5.7)</td>
<td>599.6 (9.8)</td>
<td>585.4 (10.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Deep gray matter volume (mL)</td>
<td>168.7 (3.2)</td>
<td>167.0 (2.5)</td>
<td>160.8 (4.3)</td>
<td>157.7 (4.4)</td>
<td>0.22</td>
</tr>
<tr>
<td>Total gray matter volume (mL)</td>
<td>808.6 (8.0)</td>
<td>793.5 (6.4)</td>
<td>760.5 (10.9)</td>
<td>743.1 (11.2)</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td>White matter volume (mL)</td>
<td>715.8 (11.7)</td>
<td>713.6 (9.4)</td>
<td>706.8 (15.9)</td>
<td>723.7 (16.4)</td>
<td>0.90</td>
</tr>
<tr>
<td>Ventricular CSF volume (mL)</td>
<td>34.2 (4.3)</td>
<td>38.9 (3.4)</td>
<td>37.8 (5.8)</td>
<td>48.9 (6.0)</td>
<td>0.38</td>
</tr>
<tr>
<td>Total brain volume (L)</td>
<td>1.50 (0.02)</td>
<td>1.51 (0.01)</td>
<td>1.47 (0.02)</td>
<td>1.47 (0.02)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Values are given as mean (SD), unless otherwise indicated. JND, just noticeable difference.
as described above. There was no statistically significant difference in adjusted total white matter, ventricular CSF, or whole-brain volumes among the four study groups (Table 1).

We found a statistically significant positive correlation between peroneal nerve conduction velocity and adjusted peripheral gray matter volume ($r = 0.45$, $P = 0.03$). The amplitudes in both sural and peroneal nerves were not related to peripheral gray matter volume at a statistically significant level. Peripheral gray matter volume was inversely related to NCS ($r = -0.45$, $P = 0.02$) and vibration detection threshold ($r = -0.57$, $P = 0.004$).

**FSL-VBM Assessment**
The results of the whole-brain voxelwise analyses showed lower gray matter in the primary somatosensory cortex and supramarginal gyrus in DPN subjects (Painful and Painless DPN groups) compared with subjects in the HV and No-DPN groups (corrected $P < 0.05$; Fig. 2). Reduction in gray matter volume was also found in the cingulate gyrus in DPN subjects compared with No-DPN subjects (corrected $P < 0.05$). No region was significantly smaller in either control group (HV and No-DPN groups; corrected $P < 0.05$). There were no regions of reduced gray matter volume identified when comparing the No-DPN and HV groups. Finally, we compared the Painful DPN and Painless DPN groups, and observed a significant reduction in thalamic gray matter volume in subjects in the Painless DPN group (uncorrected $P < 0.001$). Conversely, there were no regions of lower gray matter volume in subjects in the Painful DPN group compared with those in the Painless DPN group.

**CONCLUSIONS**
This is the first morphometric study to examine changes in brain structure in subjects with DPN. DPN, as the name indicates, has hitherto been considered a disease of the peripheral nerve.

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**Figure 2**—Coronal and sagittal section displaying results of whole-brain voxelwise morphometric analyses, examining between-group differences in cortical gray matter. FSL-VBM software was used for these analyses [see RESEARCH DESIGN AND METHODS for details]. A: Voxelwise differences in cortical gray matter between DPN subjects and HVs. Regions with significantly more gray matter volume loss were identified in the somatosensory cortex (Ai) and supramarginal gyrus (Aii). B: Voxelwise differences in cortical gray matter between DPN and No-DPN subjects. Regions with significantly reduced gray matter in DPN subjects were identified in the somatosensory cortex (Bi), supramarginal gyrus (Bii), and cingulate cortex (Biii). Between-group differences are represented as statistical maps color-coded on a red-yellow scale, with brighter (i.e., more yellow) regions corresponding to more significant differences. Images are presented according to neurologic convention, with right hemisphere structures shown on the right.
However, evidence is now emerging that the impact of this disorder may be more generalized than previously thought, involving the spinal cord and thalamus (8,10,35). It seems logical therefore to investigate whether other areas of the brain may also be affected by the neuropathic process. In the current study, we carefully characterized our subjects with DPN who were age-matched to control subjects. The novel finding of this study is a significant reduction in peripheral gray matter volume in subjects with established DPN (i.e., Painful DPN and Painless DPN groups) compared with subjects in the No-DPN group and age-matched nondiabetic HVs (adjusted for age and DR severity). We also found that lower levels of gray matter volume were associated with more severe neuropathy. Closer examination, using VBM techniques, suggests that the change in gray matter is mainly localized to regions involved with somatosensory perception (primary somatosensory cortex and supramarginal gyrus). There were no differences in the regional distribution of gray matter in HVs and non-neuropathic diabetic subjects. We also found greater thalamic gray matter volume reduction in subjects in the Painless DPN group compared with those in the Painful DPN group. However, this should be interpreted with caution as it was based on analysis that was uncorrected for multiple comparisons.

Minor structural changes have been reported in the brains of people with type 1 diabetes, particularly in cortical gray matter (36). In the largest study of its type so far (12), subjects with type 1 diabetes showed reductions of 4–5% in gray matter volume in several brain regions compared with healthy control subjects. The main areas involved were the posterior, temporal, and cerebellar regions of the brain. However, subjects with neuropathy (based on self-report and a review of medical records) were excluded from this study. In our study, we found a 5.4% reduction in mean gray matter volume in subjects with established DPN compared with healthy control subjects. The primary somatosensory cortex, supramarginal gyrus, and cingulate were the areas that were primarily affected by the neuropathic process in subjects with DPN. Reduction of cortical gray matter volume localized to the primary somatosensory cortex may be induced through reduced cellular activity triggered by peripheral nerve axonopathy. The biological plausibility for this hypothesis is supported by neuroimaging observations of cervical spinal cord atrophy and somatosensory evoked potential studies reporting reduced or absent amplitudes of the cortical complex in subjects with DPN (9). Furthermore, we have demonstrated continuous relations between peripheral gray matter volume NCS (predominantly large fiber assessments), which indicate a continuing loss of gray matter volume as the disease progresses. All of these points to a length-dependent injury occurring throughout the nervous system. Hence, our findings could represent the end point of neurodegeneration resulting from an accumulation of multiple pathophysiological events, such as axonal degeneration and demyelination, axonal dieback, and neuronal loss. To dissect the individual components that result in gray matter volume reduction in subjects with DPN, further longitudinal clinical and morphological studies are needed.

The findings of this study demonstrate that the neuropathic process in humans is not confined to the peripheral nerve but may also involve the brain. However, the relevance to the pathogenesis of neuropathy depends on whether there are changes in the brain of patients with early (subclinical) neuropathy. We have previously demonstrated that spinal cord atrophy appears to occur early in the neuropathic process, which, if also observed in the brain, would suggest that extensive and perhaps even irreversible damage may have occurred (8). Indeed, with this in mind, it is not surprising that, so far, the variety of therapeutic options attempted in subjects with DPN have been unsuccessful. Hence, changes in the neural architecture of the brain and spinal cord in subjects with DPN could be associated with poorer outcomes. This may suggest that conversion from normal to decreased volumes in some areas such as the somatosensory cortex or spinal cord may predict the development or irreversibility of DPN. Further longitudinal studies will be needed to test these hypotheses and offer insight into a potential point of neuroelastic inflection (i.e., reversibility) (37). These studies may also address the causality between neuropathy and loss of cortical neurons or deep white matter tracts in subjects with DPN.

Our findings suggest a similar amount of reduction in peripheral gray matter volume in subjects in both the Painful DPN and Painless DPN cohorts when compared with those in the No-DPN and HV cohorts. The only difference observed in a subgroup comparison was greater thalamic volume reduction in the Painless DPN group compared with the Painful DPN group. This finding is in keeping with our previous magnetic resonance spectroscopy report of increased thalamic neuronal dysfunction in subjects in the Painless DPN group but not in the Painful DPN group, suggesting that the preservation of thalamic neuronal function may be a prerequisite for the perception of pain in diabetes (10). In our study, caution must be applied when interpreting our subgroup comparisons between Painful DPN and Painless DPN groups, because it was based on analysis that was uncorrected for multiple comparisons. Although it increases the risk of false-positive findings, it provides an opportunity for the generation of hypotheses and the interpretation of results in the context of other work in chronic pain; we therefore believe it is potentially valuable. Clearly, with larger numbers of subjects it might be possible to identify more subtle changes in other areas between the groups.

This study has several limitations. We cannot determine causality because of the cross-sectional design. Subjects with established DPN were older (mean difference 3.3–3.6 years) compared with those in the No-DPN group. As this is a biological observational study, the age difference reflects normal clinical practice where subjects with established DPN are more likely to be older and have longer duration of diabetes. Furthermore, other microvascular and macrovascular complications of diabetes are also more likely to occur in this subgroup of patients. To account for this, we carefully characterized all DPN subjects and purposefully recruited an older cohort of HVs who were identically matched. In addition, we quantified DR severity and included it along with age as confounding variables in the analysis. However, the possible mechanisms of how these variables may influence the
outcome of our study are likely to be more generalized, affecting the whole brain and not specifically affecting the somatosensory cortical regions identified in our study. As DPN is predominantly a sensorimotor neuropathy, the most plausible explanation for these findings is a neurodegenerative process driven by this condition. There is also the inherent limitation of magnetic resonance volumetry and the caution that must be applied when interpreting the gray matter signal on MRI. Measures of brain volumetry cannot define tissue features (i.e., the cellular components) but can help to quantify the extent and magnitude of disease effects. On this basis, volumetric measures of brain structures can provide valuable insight into the underlying pathologic mechanisms of diseases. In this context, MRI measures of brain volume have been shown to be valid biomarkers of the clinical state and progression by offering high reliability and robust inference on the underlying disease-related mechanisms (38). As a result, MRI-based brain volumetry is now increasingly being used in the clinical setting to assess brain volume changes in a range of neurologic conditions (38).

Our objective was to examine the consequences of the neuropathic process on brain morphology. The data presented here suggest that there is reduced gray matter in regions involved with somatosensory perception in DPN subjects. Longitudinal prospective studies are now required to determine the natural history of brain volume reduction in subjects with DPN, and to truly dissect whether and how these consequences of diabetes are causally related. Future studies should also examine the individual components of atrophy as this may offer an insight into whether there is a point of change in cortical neuronal architecture that heralds the persistence of neuropathy. That is, could these brain structural changes in subjects with DPN be reflective of “atrophy” due to lack of stimulation from the nonfunctioning periphery? And, therefore, is it a normal process of plasticity or a pathological one? We also need to evaluate whether the distinctive pattern of gray matter volume reduction observed in subjects with DPN results in functional, cognitive, and behavioral consequences. Future studies will also need to explore the spatial relationship of the structural changes from the distal peripheral nerve to the cortex. This would include detailed neuropathy phenotyping using contact heat-evoked potentials (39) and histopathological assessments (using skin biopsy samples) (40). Finally, we have demonstrated a clear reduction in brain gray matter volume in carefully characterized patients with DPN, indicating that the neuropathic process is not confined to the peripheral nerves only but involves the whole nervous system. However, further studies are required to define the extent and temporal relationships of CNS involvement with the onset and progression of peripheral neuropathy in subjects with diabetes.

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