

Progression of brain volume reduction in a rat model of STZ-induced type 1 diabetes studied by anatomical imaging combined with voxel-Based morphometry

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Introduction Chronic diabetes affects the brain. Clinical studies have shown that poorly controlled, long-standing diabetes causes brain atrophy, as well as many other forms of brain injuries [1, 2]. In both type 1 and type 2 clinical diabetes, brain atrophy is associated with cognitive impairment [3,4]. We have shown previously that, in a rat model of uncontrolled streptozotocin (STZ)-induced type 1 diabetes, brain atrophy can be detected as early as 4 weeks after induction by high-resolution anatomical magnetic resonance imaging combined with voxel-based morphometry (VBM) analysis [5]. In this study, we measured volumetric changes in the brain of STZ-induced diabetic rats at 12 and 20 weeks using VBM. Progressive atrophy of STZ-induced diabetic brain was assessed.

Materials and methods Eight-week old male Sprague-Dawley rats, weighing 361.1±9.0g, were divided randomly into two groups with 20 animals in each group. One group received a single dose of intraperitoneal (i.p.) injection of STZ (62 mg/kg) to induce type 1 diabetes. The other group received i.p. injection of the same amount of solvent (0.01 mol/L citric acid), serving as the control. On the 3rd day after injection, the fasting blood glucose levels of the STZ-treated rats were measured. Those animals with a fasting blood glucose level < 18.8 mmol/L were excluded. All animals were scanned at the 12th week (12w) and the 20th week (20w) after induction on a 7 T/20 cm Bruker Biospec scanner under isoflurane anesthesia (1.8-2.5%, in pure O₂). A volume coil was used for RF pulse transmission, and a 4-channel phase-array coil for signal detection. High-resolution anatomical images were acquired with a RARE sequence with FOV 3.5 cm×3.5 cm, matrix size 512×384, slice thickness 0.58 mm, 54 slices, TR 5800 ms, TE_{eff} 40 ms, RARE factor 4 and 8 averages. SPM8 was used for image segmentation and co-registration. The image data from each animal were first segmented into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) probability maps, using a set of 68 μm×68 μm×68 μm GM/WM/CSF templates built in-house. The GM and WM probability maps of all animals were then entered into the DARTEL algorithm to obtain co-registered modulated GM/WM maps, followed by smoothing with a 0.2-mm FWHM Gaussian kernel. The main effects of group (STZ vs. control), age (12w vs. 20w) and group×age interaction were assessed by a voxel-wise two-way ANOVA. Statistical significance was set to a threshold of p≤ 0.005, uncorrected, and cluster size=50.

Results Compared to the control rats, the STZ-treated rats had significantly decreased body weight (Fig. 1A) and increased fasting blood glucose level (Fig. 1). At both 12w and 20w, the STZ group showed significant reduced total volume of GM, WM and the whole brain, as compared to the control group (Fig. 2). For both the control group and the STZ group, no significant age-related changes in total volume of GM/WM/whole brain were observed. Voxel-wise two-way ANOVA revealed significant effect of group×time interaction in multiple GM (Fig. 3A) and WM (Fig. 3B) regions. The GM regions involved infralimbic cortex (IL), ventral orbital cortex (VO), secondary motor cortex (M2), nucleus accumbens (NAc), primary somatosensory cortex barrel field (S1BF), primary somatosensory cortex hindlimb region (S1HL), retrosplenial cortex (RSG), caudate putamen (Cpu), hippocampus (Hip), dorsomedial periaqueductal gray (DMPAG) and ectorhinal cortex (Ect) (Fig. 3A). The WM regions involved cingulum (cg), forceps minor of the corpus callosum (fmi), optic chiasm (och), olfactory tubercle (Tu), corpus callosum (cc), external capsule (ec), ventral hippocampal commissure (vhc), stria terminalis (st), internal capsule (ic), fimbria (fi), optic tract (opt), dorsal hippocampal commissure (dhc) and deep cerebral white matter (dcw) (Fig. 3B).

Discussion The most reported atrophic brain regions associated with clinical diabetes are cortex, hippocampus and cerebellum [4,6,7]. In this study, global atrophy of the brain was observed in the STZ-treated animals at 12w and 20w, as compared to the control animals (Fig. 2). The STZ-treated animals did not show significant age-related reductions in the total volume of GM, WM and the whole brain between 12w and 20w. On the other hand, voxel-wise ANOVA revealed significant effect of group×time interaction in limbic structures, selected thalamic nuclei and somatosensory cortex, suggesting that the age-related volumetric reductions in these regions were accelerated in the STZ-treated animals relative to the control animals. These results are in general agreement with the observations in clinical diabetes. Interestingly, the STZ-induced diabetes appeared to be associated with atrophy in a brain circuitry (i.e., VO, NAc, S1 and PVG) implicated in chronic pain [8]. This is consistent with the notion that peripheral neuropathic pain is a common complication of uncontrolled diabetes.

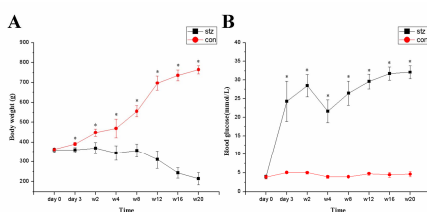


Figure 1. Compared to the control animals (Con), the STZ-treated (STZ) rats had significantly decreased body weight (A) and increased fasting blood glucose level (B). *p<0.005 compared to Con, two-sample t-tests.

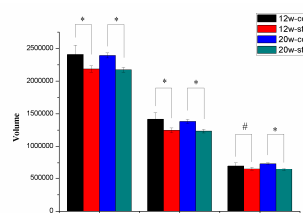


Figure 2. The STZ-treated (STZ) rats had significantly reduced total volumes of GM, WM and the whole brain at 12w and 20w. #p<0.05 and *p<0.005 compared to Con, two-sample t-tests. No significant age-related differences were found.

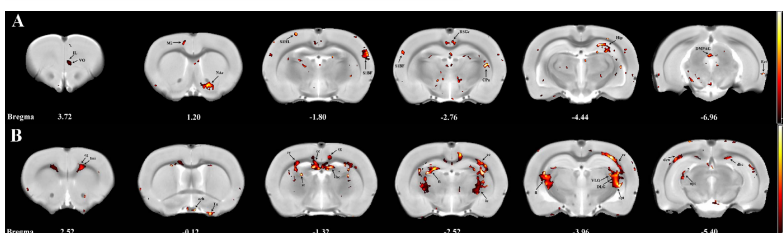


Figure 3. Voxel-wise two-way ANOVA revealed significant effect of group×time interaction in multiple GM (A) and WM (B) regions (p<0.005, uncorrected, cluster size=50).

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