Investigation of the chronic effect of streptozotocin-induced diabetes on cerebrovascular reactivity and BOLD fMRI response to electrical forepaw stimulation

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Introduction Somatosensory responses to noiceptive and non-noiceptive stimuli were altered in the peripheral nervous system (PNS) of diabetic rats (first month after STZ injection) (1) and patients (2). However, more evications suggested that the central nervous system (CNS) was also involved (3,4). For instance, the evoked potential amplitude in S1 was reduced at 8 weeks of diabetes (3). Furthermore, a resting state fMRI, which examines neuronal connectivity, study showed impairment of the attention network to external stimuli in diabetic patient (5). The goal of the current study was therefore to examine the longitudinal and chronic effect of diabetes on CNS using fMRI and CO2 challenge.

Methods Male Sprague-Dawley rats (207 to 234 g) were used. Animals were injected with 0.29 ml of buffer (N = 4) and 0.25 - 0.28 ml of streptozotocin (N = 6). 5 weeks after injection, blood glucose of the age-matched animals (AM) remained unchanged, whereas those injected with streptozotocin (STZ) increased from 102 - 136 to 347 - 525 mg/dl. Animals were initially anesthetized with 5% isoflurane, and mechanically ventilated. Isoflurane was reduced to 1.5% during MRI experiments. End-tidal CO2 was continuously monitored using a capnometer. All physiologic parameters were maintained within normal ranges. Electrical stimulation was bilaterally applied to the forepaws. Electrical stimulation of 3mA with a 12 Hz square wave with 1 ms pulse duration was applied by a constant-current stimulator. The electrical stimulation paradigm was OFF-ON-OFF-ON-OFF-ON-OFF-ON-OFF, where OFF and ON lasted for 45 and 15 s, respectively. CO2 challenge was performed with paradigm of OFF-ON-OFF-ON-OFF-ON-OFF, where OFF and ON lasted for 2 minutes with 30% balanced O2 and 2 minutes with 5% CO2 and 30% balanced O2, respectively. MRI was performed using a Bruker 7T scanner. Animals were scanned at 6, 8 and 10 months after injection. BOLD fMRI was performed using ss-GE-EPI, acquisition matrix = 96 x 96, FOV = 2.56 x 2.56 cm², TR/TE = 1 s/25 ms, flip angle = 90° and seven 1.5 mm slices. Quantitative cerebral blood flow (CBF) was measured using cASL (6) with same imaging parameters as BOLD fMRI. Because of limited variation in size and shape of the rat brain, rigid-body intergroup coregistration was performed using AIR 5.2.5. Correlation coefficient (cc) analysis was performed on a pixel-by-pixel basis to correlate fMRI signal change with the stimulation paradigm. P < 0.001 was considered as significant BOLD response. All measurements were plotted as mean ± standard deviation (SD).

Results Fig. 1 shows the cc maps of AM and STZ at 6, 8 and 10 mos after injection. Strong and localized BOLD response was observed in the primary somatosensory cortex of the forelimb region (S1FL) of AM across all time points, whereas there were localized S1FL and non-localized subcortical BOLD responses in STZ. Fig. 2 shows the % BOLD averaged from the 4 ON-OFF periods in the stimulation paradigm. Notice that there was a distinct difference between the hemodynamic of striatum of AM and STZ. In addition, there was no significant difference between the vascular response to CO2 challenge of AM and STZ across all time points.

Discussion Despite alterations of the somatosensory pathway in diabetes, our results showed that the BOLD response in S1FL of STZ was largely similar to that of AM across all time points. A noteworthy point is that BOLD fMRI is sensitive to changes in regional deoxyhemoglobin content as a result of alterations in CBF and/or oxygen consumption rate (CMRO2) (7). BOLD response is simply an indirect measure of neuronal activity. Thus our results from BOLD fMRI together with CO2 challenge experiments suggests that despite the reduction in S1 neuronal activity (which was of post synaptic origin (3), normal changes in CBF and/or CMRO2, still accompanied neural activation in chronic diabetic rats. Apart from showing normal S1FL response, diabetic rats did show widespread BOLD response in subcortical regions, but not AM. Additional structures involved in somatosensory processing such as ventral lateral nucleus (VL), ventral posterior lateral nucleus (VPL) and ventral posterior medical nucleus (VPM) (8), and structures involved in supraspinal pain processing such as medial dorsal thalamus (MDT) (9), were also activated. These results might likely suggest the normal role of chronic diabetes on the hyperactivity in somatosensory and pain processing. Similar findings were found in rats at 6 weeks of STZ injection using autoradiography (8) and electrophysiology (4). Both studies observed marked increase in activity in the VPL of diabetic rats upon peripheral stimulations. These results together with ours clearly indicated alterations in somatosensory and pain processing in the CNS, and signs of neuropathic pain. As shown in the current study and others, fMRI is a potentially sensitive and specific technique for clinical assessment of neuropathic pain (10). Apart from using BOLD fMRI, resting sate fMRI has shown promise in the diagnosis of neuropathic conditions (5,11). It is therefore valuable to perform comprehensive resting state fMRI experiments on diabetic rats in future studies to evaluate the specificity of the technique.

In summary, there was clear involvement of CNS impairment in diabetic rats resulting in alterations in the somatosensory and pain processing. One of the major implications of our results is that BOLD fMRI could be used as a non-invasive and robust means to determine whether CNS is the origin of neuropathy in diabetes.