Functional MRI of the rat spinal cord in painful diabetic neuropathy

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Introduction

The World Health Organisation predicts that by 2025 there will be 300 million sufferers from *diabetes mellitus* [1]. The incidence of neuropathy and other complications (vasculopathy, nephropathy and retinopathy) are common and increasing. With the exception of a slowing of progression of nephropathy by angiotensin-converting enzyme inhibitors, there are no treatments [2]. The aims of the current project are: to determine if functional MRI of the spinal cord can detect changes in a rat model of diabetes compared to age-matched controls and to determine if spinal fMRI can be used to detect early onset of diabetic neuropathy in this animal model.

Methods

Diabetes was induced by streptozotocin injection in specific pathogen-free Sprague-Dawley rats at four to six weeks of age. Experiments were conducted with diabetic rats (one month post-injection; n=5) and age-matched controls (n=5). The femoral artery and vein were cannulated for administration of fluids and measurement of blood gases; physiological parameters were maintained within normal parameters. Imaging was performed using a 7 T Bruker Avance console (Bruker, Germany) with the rat placed supine with the lumbar enlargement centred over a surface coil tuned to 300 MHz. Fast spin-echo images were acquired using a 2.5 cm field of view, 60 ms echo time, 128 x 64 matrix and six 2 mm thick slices transverse to the spinal cord corresponding to lumbar segments L1 to L6. Images were gated to the respiration (TR approximately 3 s). Sixty images were acquired with alternating blocks of 6 rest and 6 activation periods for the electrical stimulation (3 Hz, 0.3 ms, 15V; right hind paw). A total of 200 repetitions were acquired for formalin experiments, with injection of formalin after the 6th image (50 µl sc.; 0.2% left and 5% right hind paw in separate experiments). Data were analyzed using IDL software written "in house". Data were directly correlated to the paradigm at $p \leq 0.05$ and $p \leq 0.01$.

Results

Diabetic animals were smaller and hyperglycemic, and blood pH was lower than in control animals. Very little fMRI activity ($p \leq 0.01$) was observed in the diabetic animals during electrical stimulation. The activity was mainly located in the right dorsal horn of spinal cord segments L3 to L5 (Figure 1b). More activation was found in the control animals, mainly in the dorsal horn at spinal cord segments L2 to L5. Activity was also observed in the ventral horn of control animals (Figure 1a). During 0.2% formalin experiments, functional activity ($p \leq 0.05$) was observed in control animals mainly in the dorsal horn of L2 to L5 contralateral to the injection site. In diabetic animals, activations were observed in both the ipsilateral and contralateral dorsal horns and in the right ventral horn (Figure 1c,d). The time courses for both the electrical and 0.2% formalin experiments are shown in Figure 2. Percentage signal changes for diabetic animals were slightly less than for control animals during noxious electrical stimulation. Diabetic animals showed greater signal changes during both 0.2% and 5% formalin injection. Activity was observed in the ipsilateral and contralateral dorsal horns for both groups of animals following 5% formalin injection.

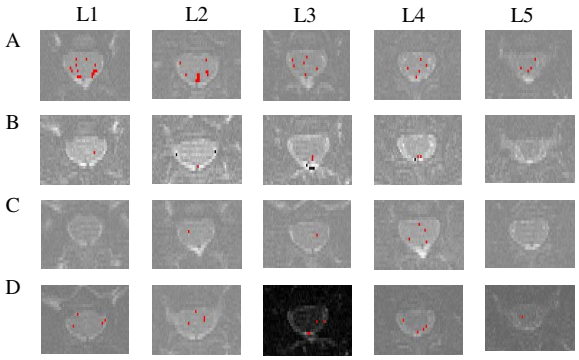

Figure 1. Examples of activation maps ($p \leq 0.01$) for A) control and B) diabetic rats during electrical stimulation and after injection of 0.2% formalin into the right hind paw ($p \leq 0.05$) for C) control and D) diabetic rats. The right side of the image is the right side of the animal; top is ventral and bottom dorsal.

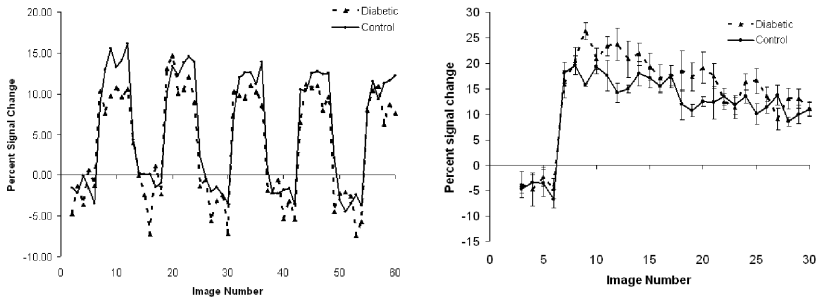

Figure 2. Activity-related signal change in the spinal cord of diabetic (n=4) and control (n=4) rats during electrical stimulation (($p \leq 0.01$; left) and 0.2% formalin injection ($p \leq 0.05$; right) (x ± SE).

Discussion and Conclusions

Both groups of animals demonstrated activity in the right dorsal horn during noxious electrical stimulation, consistent with previous spinal fMRI studies [3,4] and spinal cord physiology. FMRI activity was observed in the lumbar spinal cord enlargement ipsilateral to the side of the stimulus. Diabetic animals had much less activation and lower percentage signal changes than controls (Figures 1 and 2). This may reflect the beginning stages of neuronal fibre dysfunction in diabetic rats.
Noxious stimulation with 5% formalin injection into the left hind paw demonstrated greater percentage signal changes, immediately post-injection, in diabetic compared to control rats. Similarly, injection of 0.2% formalin in the right hind paw showed significantly less percentage signal changes within the first 7-8 images following formalin introduction in control animals compared to diabetic rats. Greater ipsilateral dorsal horn activity was also observed in diabetic rats following 0.2% formalin injection. Behavioural studies have shown that control animals do not demonstrate the flinching response at 0.2% formalin, while it is observed in diabetic animals [5]. The fMRI results support these findings, demonstrating the hypersensitivity in diabetic rats. The additional regions of functional activity are expected and may result from neuronal interconnections. There may also be slight swelling at the site of the subcutaneous injection as well as slight movement of the paw resulting in ventral horn activity in the fMRI. The results are very promising and suggest that fMRI may be useful in pre-symptomatic screening for diabetic sensory neuropathy.

References

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