# Application of the MR Diffusion Anisotropy Imaging for the Assessment of MPEP Neuroprotection Effects on the Rat Spinal Cord Injury *in vivo*

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**Synopsis** Diffusion anisotropy imaging (DAI) of the rat spinal cord after contusion using weight-drop method was used to study the neuro-protecting effect of 2-methyl-6-(phenylethynyl)-piridine (MPEP) – an mGlu5 receptor antagonist. 18 rats were investigated, divided into 3 groups of 6 animals: a control group with laminectomy, a reference group with injury and a test group with injury and MPEP. DAI was performed at 4.7 T at 1h, 24h, 48h and 7d after the injury. DAI results confirm positive effect of MPEP on the limitation of secondary exitotoxic injury in the spinal cord.

**Introduction** Water diffusion tensor imaging (DTI) is a well-established, non-invasive method of visualization and quantitative assessment of the injury of spinal cord tissues [1,2]. Diffusion anisotropy imaging (DAI) requiring half time of DTI may be used successfully in structures having high symmetry like the spinal cord [3]. Mechanical injury creates complicated changes in structure and function of spinal cord tissues. Damage of nerve fibers and blood vessels gives rise to ischemia, bleeding and non-equilibrium of different biochemical processes leading to an avalanche of secondary processes resulting in permanent damage of the spinal cord. Excitatory amino acids (EAA) appear in abundance in the intercellular space after the trauma stimulating ionotropic and metabotropic glutamate receptors, generating the secondary damage to GM and WM. Ionotropic glutamate receptors coupled to the ion channels are: NMDA, AMPA and kainate. Metabotropic glutamate receptors (mGluR) are linked to G-proteins. We hypothesized that 2-methyl-6-(phenylethynyl)-piridine (MPEP) – an mGlu5 receptor antagonist, used previously as an antidepressant drug [4], may limit secondary excitotoxic injury after spinal cord trauma.

In this paper we present results of our studies of MR water diffusion anisotropy imaging (DAI) of neuroprotecting effects of MPEP after spinal cord trauma (SCT) on a rat model following the long-term development of injury up to 7 days. Results were compared with histopathology.

**Materials and Methods.** 18 male Wister rats of 250 g to 300 g weight divided into 3 groups were used. A laminectomy at the Th12 spine level was performed and the SCT was induced using a dynamic weight-drop. MPEP was injected intraperitonealy before the injury and at 24h and 48h after the injury at a dose of 30 mg/kg. Rats were anesthetized to a surgical depth with halothane and were maintained at 37° C using water blanket. An ECG and motion detector was placed on their chest to synchronize the MRI system to the animal breath rate. Each rat was measured 4 times at 1h, 24h, 48h and 7d after trauma.

MR DAI experiments were performed at 4.7T with a MARAN DRX console, using a SE sequence with diffusion gradients applied parallel and perpendicular to the spinal cord. Dedicated inductively coupled probes were used to record MR images of 128 x 128 with a FOV of 2 cm, 3 slices of 1.6 mm thick positioned at the injury center, at 2.8 mm and 5.6 mm rostrally and with gradient b-factors up to 1550 mm$^2$/s. All experiments were ECG and breath triggered. Each rat was measured 4 times at 1h, 24h, 48h and 7d after the trauma. Data were analyzed using IDL based software developed in-house. Longitudinal diffusion $D_L = D_{ZZ}$, transverse diffusion $D_T = (D_{XX}+D_{YY})/2$, isotropy index $ID = D_T/D_L$ and anisotropy index $AI = (DL-DT)/(DL+DT)$ were determined for 11 selected ROI's in the white and gray matter of the spinal cord. Behavioral observations of all rats were carried out during 7 days using BBB and Tarlow scales.

**Results and Discussion.** Good quality MR images free from any motion artifacts were obtained from control and injured spinal cord of the living rat. Axial DW images for slices 1 and 3 for an injured rat and treated with MPEP recorded 24h after the trauma, are shown in Fig. 1. DW images and ADT maps for sagittal and axial slices. Sagittal DW images and ADT maps delineate the traumatic region and its development in time very well, whereas axial DW images and ADT maps show development of injury in different anatomical regions. Detailed analysis of data from 5 ROI in the GM and from 6 ROI in the WM gives quantitative description of injury development in time. Fig. 2 presents time development of anisotropy index AI for slices 1 and 3 for WM and GM, averaged over all ROI's, for injured rats without and with MPEP. In the central slice a significant decrease of AI is observed in WM and GM for the injured rats, whereas AI changes in rats with MPEP are limited. For the slice at 5.6 mm from the injury center AI is constant for rats with MPEP, equal to values for control-uninjured rats. These results are confirmed by behavioral observations and by subsequent histopathology [5].

**Conclusion** Our results show that MPEP – an mGlu5 receptor antagonist is an effective neuro-protecting agent in SCT injury and that *in vivo* DAI of the spinal cord in a rat model can be used for drug testing.

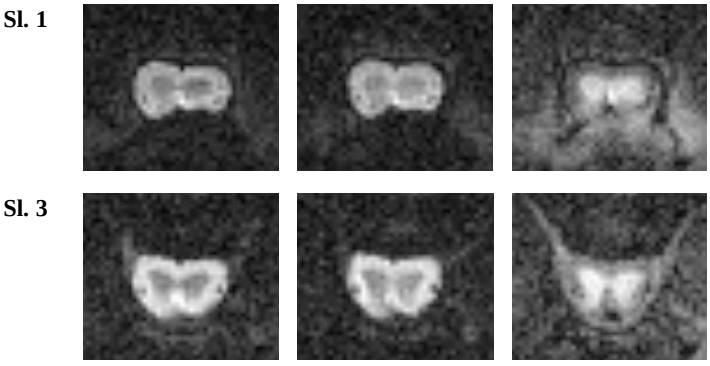


Fig. 1. Axial DW images of the injured spinal cord of a rat with MPEP recorded 24 h after SCT from Sl.1 – at 0 mm and Sl.3 – at 5.6 mm.

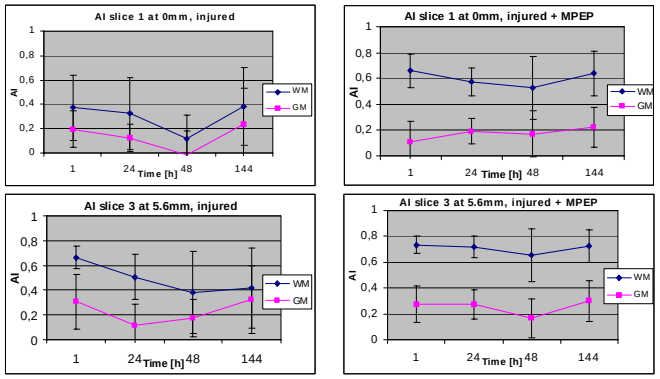


Fig. 2. Time dependence of anisotropy index AI in WM and GM for injured spinal cord and injured with MPEP, for Sl.1 – at 0 mm and Sl.3 – at 5.6 mm.

**References:**

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