Multimodal MR evaluation of experimentally induced apoptotic neuronal death in the rat brain

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Introduction:

Apoptotic cell death is the leading cause of neuronal loss after brain insults and in neurodegenerative processes [1,2]. Apoptosis is a 'programmed' cell death representing typical morphological changes such as nuclear pycnosis with internucleosomal degradation of chromatin and shrinkage of the cell body with maintenance of organelle integrity [3]. Despite the importance of apoptosis in brain pathologies, in vivo diagnostic of this process is still not developed. The aim of this work was to evaluate the possibility of using non-invasive MR techniques to detect apoptotic neuronal death in the rodent cerebral cortex. For this purpose, we exploited a new, highly reproducible neuronal ablation model utilizing a diphtheria toxin (DT) and diphtheria toxin receptor (DTR) system to induce synchronized apoptotic cell death of selective cortical neuron populations in rats [4]. Neurons are eliminated by apoptosis and no signs of necrosis, autophagy and disruption of the blood brain barrier could be detected. To assess this model, an in vivo multimodal MR protocol at 9.4T with ^{1}H-Magnetic Resonance Spectroscopy (MRS) and Diffusion Tensor Imaging (DTI) was used.

Materials and Methods:

14 Wistar rats from 3 different litters were used. Diphtheria toxin (DT) is known to induce apoptosis in cells via diphtheria toxin receptor (DTR). As such, DTR was genetically introduced into targeted neurons. For this purpose, layer IV neural progenitors were electroporated unilaterally with plasmids carrying green fluorescent protein (GFP) and DTR-containing constructs at embryonic day 16. At post-natal day 15 (P15), an injection of diphtheria toxin (DT) was performed to induce apoptosis of targeted neuronal population. All MR experiments were performed on an actively-shielded 9.4T/31cm magnet (Varian/Magnex) equipped with 12-cm gradient coils (400mT/m, 120µs) with a quadrature transceive 20-mm surface RF coil. 5 days after DT injection, after automatic FASTMAP [5] shimming, spectra acquisition on a volume of interest of 1.5×1.5×2.5 mm^3 within the ipsilateral and contralateral cortices were performed using an ultra-short echo time (TE/TR = 2.7/4000 ms) SPECIAL spectroscopy method [6]. 22 series of FIDs (16 averages each) were acquired, individually corrected for frequency drift, summed together and corrected for residual eddy current effects using the reference water signal. Proton spectra were analyzed with LCModel [7]. DTI acquisition was performed using a semi-adiabatic double spin-echo sequence [8] with the following parameters: Icosahedral 21 directions diffusion gradient sampling scheme (b = 1000 s.mm^{-2}), FOV = 23 × 15 mm^2, matrix size = 128 × 64 zero-filled to 256 × 168, 8 slices of 0.8 mm thickness in the axial plane, 8 averages with TE/TR = 42/2000 ms. Immediately after MR experiments, brains were perfused with 4% PFA; immunostained for GFP, Iba1 (marker for microglial cells), GFAP (marker for astrocytes) and stained by Hoechst (nuclear staining). The extent and position of apoptotic cells were delineated on GFP images and ROIs were manually delineated in the known apoptotic areas as well as in the contralateral position (in external capsule (EC) and cortex (Cx)). Diffusivity values (MD, $D_{//}$ and $D_{\perp}$) as well as fractional anisotropy (FA) were derived from the tensor and averaged in these ROIs. A Wilcoxon non-parametric paired test was used to compare statistically values between ipsilateral and contralateral ROIs, significance was reached when P<0.05.

Results:

Histological analysis (Fig. 1B,C): Electroporated GFP-positive neurons expressing receptor to DT were observed principally in layer IV of somatosensory cortex. Five days after initiation of cell death most of the fluorescent cells were still in place, however, apoptotic cells showed striking morphological changes including shrinkage of the cell bodies, fragmented and shrinked processes, presence of the pycnotic nuclei. Neuronal death induced an activation of microglial cells (labeled with Iba-1 antibodies) and a development of the reactive astrogliosis (detected with GFAP antibodies).

MRS (Fig. 1A): very good spectral quality was achieved in the current study, as judged from water linewidth, obtained with FASTMAP ranging from 8 Hz to 10Hz. Due to very thin cortical structure in the rat pup brain, MRS was performed on a very small volume of 12 µl placed on the cortex. On the overall study SNR was equal in average to 24 ± 2. Such consistent data was subjected to spectral analysis and absolute quantification by LCModel provided the concentration of 18 metabolites defined as the "neurochemical profile". In the ipsilateral cortex, significant decrease of [NAAG] (1.0±0.2 *vs.* 0.7±0.2mM/g, P=0.008), [NAA+NAAG] (9.2±1.0 *vs.* 8.4±0.5mM/g, P=0.04) and [Glu]/[Gln] (2.8±0.3 *vs.* 2.3±0.4mM/g, P=0.01) was observed whereas [Ins] was significantly increased (1.9±0.4 *vs.* 2.5±0.3mM/g, P=0.01).

DTI (Fig. 1D-G.): the ipsilateral cortex exhibited significantly lower $D_{//}$ and a tendency to higher $D_{\perp}$ leading to a significant decrease of FA. In the external capsule a significant increase of all the diffusivity values (i.e. MD, $D_{//}$ and $D_{\perp}$) was observed in the ipsilateral side.

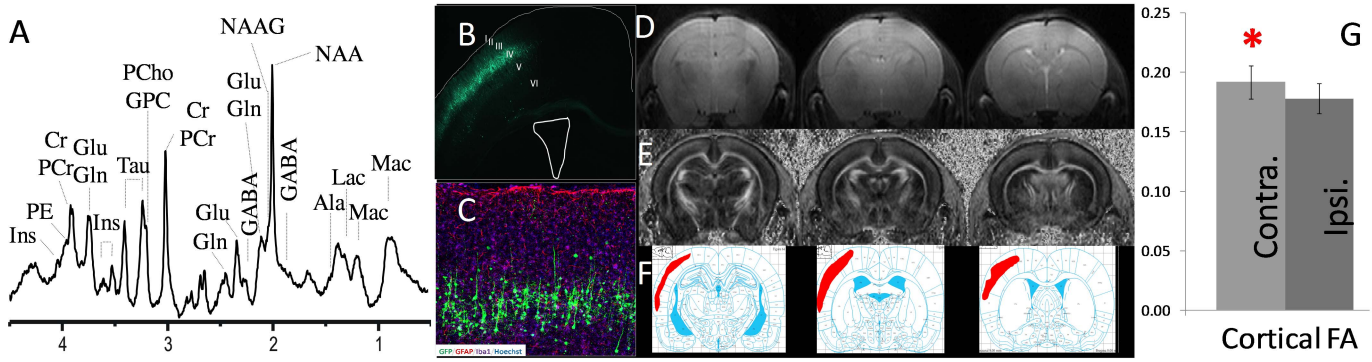

Figure 1: typical spectrum acquired in the cortical lesion (A) at 9.4T. Electroporated cells in layer IV of the somatosensory cortex (B). Confocal image of the lesioned region on 5-th day after DT injection where dying cells labeled by GFP (green), reactive astrocytes marked by GFAP (red), activated microglial cells stained with Iba1 (purple); nucleus stained by Hoestch (blue) (C). Typical anatomic MR images (D) and FA maps (E) as well as apoptotic regions, observed by histological analysis, overlaid on an atlas to position ROIs of MR analysis (F). Histogram of FA values in the contralateral and ipsilateral cortices (G).

Discussion and conclusion:

Here we used in utero electroporation to express of DTR in rat that confers DT sensitivity to specific populations of cortical neurons. These neurons die by apoptosis and remain in place for at least five days after DT injection. A decrease of [totalNAA] in the apoptotic region was observed, providing evidence of neuronal loss and/or suffering. In the apoptotic cortex, lesion site became more isotropic, indicating the tissue disorganization probably due to neuronal outgrowth degeneration, which has been confirmed by confocal analysis of the dying cells. Observed decrease of [Glu]/[Gln] ratio indicated dysfunction of the Glu-Gln cycle which characterizes crosstalk between astrocytes and neurons and is important for the activity of GABA-ergic synapses. [Ins], a glial tissue integrity marker, was increased, in relation with the glial reactions observed in the apoptotic region. In conclusion, changes induced by apoptotically dying neurons 5 days following DT injection were detected by using non-invasive multimodal MR techniques. Further experiments are in progress to assess different stages of apoptosis as well as the long-term consequences on cortical functions.

References

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