Pharmacological Manganese-Enhanced MRI (phMEMRI) without osmotic breakdown of the Blood Brain Barrier

A. Gozzi[1], A. Schwarz[1], V. Crestan[2], and A. Bifone[3]

[1]Neuroimaging, GlaxosmithKline Medicine Research Centre, Verona, Italy, [2]Laboratory animal science, GlaxosmithKline Medicine Research Centre, Verona, Italy, [3]CPDM, GlaxosmithKline Medicine Research Centre, Verona, Italy

Introduction

Functional MEMRI methods can be applied to map brain regions that exhibit increased synaptic activity, owing to the ability of Manganese ions (Mn^{2+}) to enter cells through voltage-gated Ca^{2+} channels [1]. The application of this approach to pharmacological studies would enable the investigation of the effects of drugs on neuronal activity, and would complement phMRI approaches that measure haemodynamic responses as a surrogate. However, the practical implementation of this technique is limited by the poor permeability of the Blood Brain Barrier (BBB) to Mn^{2+}, and invasive approaches (e.g hyperosmotic breakdown of the BBB) have been used to map MEMRI signal responses in brain parenchymal structures [2]. However, hyperosmotic challenges are of limited use for pharmacological studies as they alter the pharmacokinetics of the drug and its penetration into the brain.

A few MEMRI studies have been conducted without BBB osmotic breakdown, and have demonstrated the possibility to detect changes in neuronal activity in periventricular auditory structures [3] and hypothalamic areas where the BBB is less tight [4]. Recently, we have observed weak but reproducible MEMRI signal increases in the rat cortex upon continuous infusion of $MnCl_2$, thus suggesting that some degree of BBB permeability to Mn^{2+} exists also in cortical regions. Here we have investigated whether this intrinsic permeability can be exploited to map drug-induced activity in the rat brain with MEMRI in the presence of an intact BBB. The feasibility of the protocol was assessed using acute challenge with amphetamine, a psychostimulant drug that has been shown to induce functional MRI responses and changes in glucose metabolism in discrete brain structures [5].

Methods

Experiments were carried out in accordance with Italian regulations governing animal welfare and protection. Protocols were also reviewed and consented to by a local animal care committee, in accordance with the guidelines of the Principles of Laboratory Animal Care (NIH publication 86-23, revised 1985). **Animal preparation**: Male Sprague-Dawley Rats (347±8g) were anaesthetised with isoflurane and prepared as described previously [6]. Left and femoral veins were cannulated to allow drug challenge administration and continuous infusion of $MnCl_2$. Arterial blood pressure and blood gases were measured through left femoral artery. Image acquisition was performed under 1% isoflurane anaesthesia, neuromuscular blockade and artificial ventilation **Experimental groups**: $MnCl_2$ (12.5 mg/ml, 3 ml/hr) was infused continuously over 40 min; midway through this time-window rats received an i.v. injection of either saline (1 ml/Kg; n=5) or amphetamine-sulphate (3 mg/Kg; n=5). The infusion of $MnCl_2$ was started after a 10-min period of baseline acquisition. **MEMRI acquisition protocol:** MEMRI time series data were acquired on a Bruker Biospec 4.7T system using a T1-weighted FLASH sequence[7] (matrix 256x256; FOV 40mm; slice thickness 1mm; 24 contiguous coronal slices; α=90, TE=4.9; TR=400ms; δt=120s). **Data analysis:** time series data were spatially normalised to a reference study template [8]. Signal changes were normalised to pre-injection baseline. Individual subject response amplitude maps were calculated within the framework of the general linear model using FEAT (FMRI Expert Analysis Tool) Version 5.63, part of FSL (www.fmrib.ox.ac.uk/fsl) and using a model function identified by Wavelet Cluster Analysis (WCA)[9]. These MEMRI maps were compared with relative cerebral blood volume (rCBV) maps previously obtained in our lab under identical experimental conditions [10].

Results and Discussion

A summary of the results is illustrated in figure 1. Consistent with recent studies performed in our lab, infusion of $MnCl_2$ produced weak but significant signal increases in brain parenchyma. Amphetamine challenge elicited bilateral MEMRI signal increases in discrete cortical and subcortical structures, including the orbitofrontal, insular and visual cortices, and selected striatal and thalamic nuclei. The MEMRI pattern showed a high degree of correspondence with the rCBV response [5], and with the changes in glucose metabolism induced by acute amphetamine challenge [11]. Interestingly, the MEMRI signal remained sustained after the infusion of $MnCl_2$ was stopped, consistent with the hypothesis that Mn^{2+} ions, once within the CNS, are rapidly internalised [1]. These data demonstrate the feasibility of functional MEMRI without the need of invasive procedures to breakdown the BBB, and pave the way to the use of this approach to pharmacological studies.

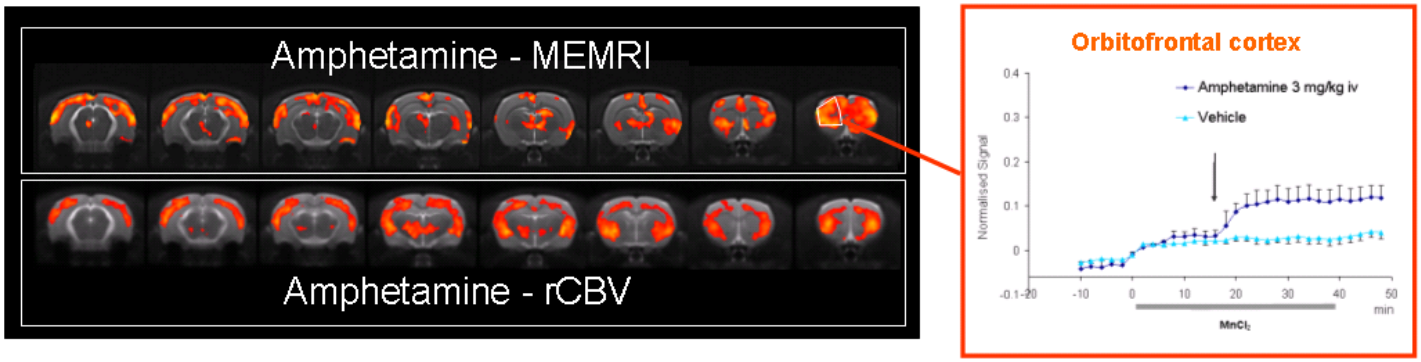

Figure 1. Left: Anatomical distribution of the MEMRI (top, p<0.01) and rCBV (bottom, p< 0.00001) signal changes produced by an acute amphetamine challenge, versus vehicle baseline. The rCBV map was previously produced and published by our laboratory [10]. Yellow/Orange indicates statistically significant increase in signal versus baseline. The maps were thresholded using a corrected cluster significance threshold of p=0.05 Right: time-profile of the MEMRI signal in a representative brain region.

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