Characterization of rat spinal cord vasoreactivity using arterial spins labelling at 9.4 T

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Target audience: Scientists interested in the metabolism and the hemodynamics of the central nervous system.

Purpose: Spinal cord blood flow (SCBF) is an important parameter for the characterization of different spinal cord (SC) pathophysiology. In particular, measurement of spinal cord vasoreactivity might provide valuable information in conditions in which the delivery of nutrients to the spine is stretched, such as in animal models of Multiple Sclerosis, namely Experimental autoimmune encephalomyelitis (EAE). In particular, one of the newest hypotheses within the field of EAE is that the primary lesions appear through an imbalance between oxygen delivery and utilisation, leading to a transient hypoxic condition [1]. To test such a hypothesis, an assessment of oxygenation and perfusion in the spinal cord of EAE rats would help in the investigation on the original pathophysiological cascade of events leading to the formation of spinal lesions. In this work, we used a recently optimised Arterial Spin Labelling (ASL) technique [2] to assess the feasibility and reproducibility of measuring vasoreactive changes in SCBF to a carbogen challenge in healthy rats.

Methods: Experiments were performed on Dark Agouti rats (n=4) on a 9.4T Agilent scanner (Agilent Technologies, CA, USA) using transmit volume coil (Ø=72 mm) and two elements receive array coil (Rapid Biomedical). Each rat underwent hypercapnic challenge (carbogen: 5% CO2 and 95% O2) after baseline measurements (medical air: 21% O2 and 79% N2). The two gases mixtures were delivered at a constant flow (1.5l/min) through a dedicated nose cone. Physiology (pulse, oxygen saturation) was monitored using a pulse oximeter attached to the hindlimb. During the scanning, the animals were maintained under 2% isoflurane and spontaneous respiration. The concentration of isoflurane was regulated to obtain regular breathing at a frequency of 30-40 breaths per minute. The applied presaturation FAIR ASL method is detailed in [2]. Briefly, after a global shimming and based on fast localization imaging, the animal is positioned within the volume coil. The surface coil was positioned at the lumbar level. The recovery time τ after a non-selective pre-saturation was set to 3.2 s. A hyperbolic secant AFP RF pulse was applied with and without the slice selection gradient (Slab thickness=4mm, BW=10 kHz, T=10 s, TI=1.65s). The equilibrium magnetisation, the inversion efficiency, and the tissue apparent longitudinal relaxation were measured under both normocapnia and hypercapnia using a slice selective inversion recovery acquisition (τ=10s, TI = 0.1, 1, 2, and 9s). An inplane spatial resolution of 125x125um^2 was performed using four shots EPI readout technique. For each measurement, air and cargoben, TE/TR=20/5015ms, NEX=12, and the acquisition time was 25 min. The breathing was checked permanently during the scanning in order to reduce the movement artefacts. The data are not saved when either the inversion or the readout are not performed during the quiescent phase of the respiratory plateau. The acquisition parameters were adjusted during the scanning.

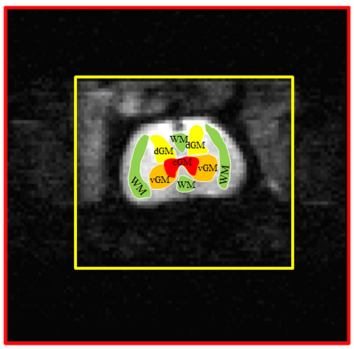

Fig1 Anatomical image of rat spinal cord at lumbar level acquired with reduced FOV (red rectangle). The yellow rectangle corresponds to the selected volume of interest using adiabatic RF pulses. The selected ROIs within the dorsal GM (dGM), ventral GM (vGM), central GM (cGM), and WM are depicted.

Results: Typical SCBF maps under both normocapnia normoxia and hypercapnia hyperoxia obtained at the lumbar level of the rat spinal cord are depicted at Fig2. On these images, the characteristic butterfly shape of the GM is clearly visible. The averaged SNR within the GM and the WM were 4.5 and 2.5, respectively. The quantitative measurements within the ROIs depicted in Fig1 are summarized in Table 1. Within the GM, the averaged perfusion under isoflurane mixed with air and with carbogen was 99.4±6.1 ml/100g/min and 117.6±10.8 ml/100g/min, respectively. However, within the WM it was 38.9±7.3ml/100g/min and 51.4±7.7ml/100g/min under air and carbogen, respectively. The group standard deviations of perfusion values measured in GM and WM were less than 11% and 19%, respectively. Significant increase of the SCBF within the GM due to the hypercapnia was noticed (18.2±6.6 %, p<0.05). The vasoreactivity was different between different areas with the GM. The highest increase was noticed within the dGM (29.9±5.1 %, p<0.005). There was an increase but non-significant within the WM.

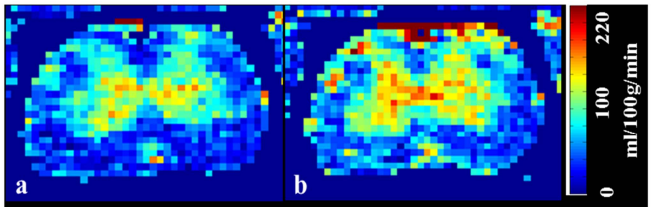

Fig.2 Absolute SC blood flow maps acquired under anaesthesia mixed with air (a) and carbogen (b)

	Air		Carbogen		
	SCBF (ml/100g/min)	**Var(Group) (%)**	**SCBF (ml/100g/min)**	**Var(Group) (%)**	**ΔSCBF (%)**
dGM	87.4±8.7	9.9	113.6±12.5	11.0	29.9±5.1 **
vGM	103.9±5.4	5.2	118.7±10.7	9.0	14.1±7.5 *
cGM	122.4±6.0	4.9	138.4±5.8	4.2	13.2±1.8 **
tGM	99.4±6.1	6.1	117.6±10.8	9.2	18.2±6.6 *
tWM	38.9±7.3	18.9	51.4±7.7	15.0	35.1±21.3

Table 1 SCBF under normcapnia normoxia (air) and hypercapnia hyperoxia (carbogen) and relative variation in SCBF within each region of interest (** p<0.05, *<0.005).

Discussion: High spatial resolution SCBF maps were obtained by using an efficient respiratory gating and adapted slice selection approach. The quantified values in our study are much lower than those reported in the only published mice SCBF paper ($SCBF_{GM}$=285±27ml/100g/min and $SCBF_{WM}$=100±32 ml/100g/min) [3]. Compared to the rat brain cortex (110-150 ml/100g/min), the averaged $SCBF_{GM}$ in our study might appear slightly lower [4,5]. The estimated increase of SCBF was in agreement with previous studies [6]. Interestingly, different areas of the spinal cord show different reactivity, with a high degree of reproducibility. This was clearly not expected, as vasoreactivity in the brain is usually rather equivalent through the brain [4]. Some of these differences might have to do, within the spinal cord, with the particular orientation of perforating vessels, and the possible geometrical disposition of the GM, surrounded by WM, as opposed to brain. Possibly, this difference in vasoreactivity could provide a hint as to where EAE lesions usually appear, depending on the capacity of the different tissues to maintain and increase delivery of oxygen under hypermetabolic conditions.

Conclusion: For the first time, the feasibility of measuring vasoreactive changes in SCBF to a carbogen challenge in healthy rats was shown. Different areas of the spinal cord show different reactivity with a high degree of reproducibility. These preliminary results highlight the potential of the developed technique in the investigation of the pathogenesis of neuroinflammatory diseases such as Multiple Sclerosis.

Reference: [1] Davies et al. AnnNeurol 2013 ; [2] Tachrount et al. ISMRM 2015 ; [3] Duhamel MRM 2009 ; [4] Zheng NMBiomed 2010 ; [5] Pell MRM 1999 ; [6] Donahue JCBFM 2013.