CMRO$_2$ quantification by direct ^{17}O MRI at 7 T in the macaque brain: assessment of energy metabolism impairment *in vivo*

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TARGET AUDIENCE This work should be of interest for researchers studying brain energy metabolism.

INTRODUCTION

Direct oxygen-17 (^{17}O) MRI is a promising tool for mapping the cerebral metabolic rate of oxygen consumption (*CMRO$_2$*) *in vivo* through the detection of the reduction of $^{17}O_2$ into ^{17}O-labelled water ($H_2{}^{17}O$) [1]. As such, this technique could allow the monitoring of brain energy metabolism in normal and pathological conditions. However, it suffers from relatively low SNR, the cost of the enriched ^{17}O gas and the difficulty to accurately quantify *CMRO$_2$*. Recently, measurements were performed in rodents and in a human volunteer [2, 3] and a new three-phase metabolic model of $H_2{}^{17}O$ production and clearance was proposed [3]. In this study, we developed a simple gas delivery system and conducted direct ^{17}O imaging experiments *in vivo* in the brain of one healthy macaque monkey and one macaque monkey undergoing chronic oxidative stress induced by 3-NP (3-nitropropionic acid), a mitochondrial toxin inhibiting succinate dehydrogenase in the TCA cycle and used as a model for Huntington's disease [4].

MATERIALS AND METHODS

In vivo experiments Experiments were conducted on a 7 T Agilent primate MRI scanner. A ^{17}O loop coil was used for RF transmission and reception. Acquisitions were performed before, during and after $^{17}O_2$ inhalation using a 3D pulse-acquire CSI sequence (TR/TE=4.5/0.36 ms, 18.5 s per image, 8.75 mm isotropic voxels on a 16×16×16 data grid, total scanning time of 35 minutes). Experiments were carried out on two macaque monkeys (*Macaca fascicularis*) anesthetized by an i.v. propofol infusion: one healthy macaque monkey (control) and one 3-NP treated macaque monkey (chronic systemic injection).

^{17}O gas delivery During the experiments, the animals were intubated and connected to a three-way stopcock to efficiently switch from a poorly enriched $^{17}O_2$ gas (natural abundance) to a 70% enriched $^{17}O_2$ gas (NUKEM isotopes GmbH, Germany). A closed-loop breathing circuit, containing a CO_2 absorber to maintain longer $^{17}O_2$ inhalation, was filled with $^{17}O_2$ gas before the experiments. The system, displayed in *Figure 1*, allowed the animals to spontaneously breathe the $^{17}O_2$ gas over a period of 10 minutes.

Data analysis and modeling k-space was filtered using a 3D Hanning window. The signal from each voxel was then quantified by measuring the area under the $H_2{}^{17}O$ resonance peak. The signal collected before $^{17}O_2$ inhalation was averaged, converted into moles of $H_2{}^{17}O$/g of brain tissue and used to scale the entire $H_2{}^{17}O$ dynamic. A three-phase metabolic model, proposed by Atkinson *et al.* [3], was used to quantify *CMRO$_2$*. Basically, the amount of $H_2{}^{17}O$ in each voxel ($M_v^{H_2{}^{17}O}$) depends on (i) the conversion of $^{17}O_2$ into $H_2{}^{17}O$ in the mitochondria (*CMRO$_2$*), (ii) the clearance of $H_2{}^{17}O$ from brain tissue to the arteriovenous system (with a rate constant K_L) and (iii) the gain of $H_2{}^{17}O$ due to the transfer of $H_2{}^{17}O$ produced in the entire body from the blood to the brain tissue (with a rate constant K_G). This model is described by the following equation: $dM_v^{H_2{}^{17}O}/dt = 2 * CMRO_2 * A^{17O}(t) - K_L * M_v^{H_2{}^{17}O}(t) + K_G * B_v^{H_2{}^{17}O}(t)$ where A^{17O} and $B_v^{H_2{}^{17}O}$ are the amount of arterial ^{17}O and $H_2{}^{17}O$ in the blood in excess of natural abundance. A^{17O} depends on the pulmonary arteriovenous difference fraction (F_{AV}=0.25) and the mean blood circulation time (T_C=20 s). $B_v^{H_2{}^{17}O}(t)$ is proportional to the amount of A^{17O} converted into $H_2{}^{17}O$ in the entire body up to time *t*. When fitting $H_2{}^{17}O$ dynamics, three parameters were adjusted in each voxel: *CMRO$_2$*, K_L and K_G.

RESULTS AND DISCUSSION

Control monkey The good quality of the signal allowed us to monitor the $H_2{}^{17}O$ signal dynamics in each brain voxel. Signal dynamic in one voxel is reported in *Figure 2*. When fitting the experimental data, *CMRO$_2$* values were below 1.2 µmole/g of brain tissue/min, which is lower than expected values estimated from previous TCA cycle rate (V_{TCA}) measurement in the monkey brain ($CMRO_2 \sim 3V_{TCA} \sim 1.8$ µmole/g of brain tissue/min, [5]). This difference can be explained by different assumptions made when modeling the experimental data. We arbitrarily fixed F_{AV} and T_C to values measured in humans [3] and assumed that the proportion of cerebrospinal fluid (CSF) was negligible. A decrease in F_{AV} and considering that the voxel encloses some proportions of CSF would lead to an increase in *CMRO$_2$*.

3-NP treated monkey This animal presented a bilateral 3-NP striatal lesion. We selected an axial slice that includes the lesion on both sides and compare the *CMRO$_2$*, K_G and K_L in three regions of interest in both the control and treated monkeys. As shown in *Figure 3* and reported in *Table 1*, we found a 35-42% decrease in *CMRO$_2$*, a 40-60% decrease in K_G and 11-42% decrease in K_L. Note that, when imposing the same K_L in both the healthy and treated monkeys (there is no obvious reason why the clearance from brain tissue would be reduced under 3-NP treatment), a similar decrease in *CMRO$_2$* and K_G was found. Decreased K_G may reflect a general decrease of oxidative metabolism and $H_2{}^{17}O$ production in the entire body. Measured *CMRO$_2$* decrease is consistent with previous works performed in our group with ^{13}C NMR spectroscopy [6] and with the effect of 3-NP intoxication (inhibition of succinate dehydrogenase in the mitochondria) [4].

CONCLUSION

In the present work, direct ^{17}O MRI experiments were performed for the first time in the monkey brain. The quality of the data allowed us to measure *CMRO$_2$* voxel-by-voxel in the brain of one healthy and one 3-NP treated macaque monkeys. *CMRO$_2$* values were lower than expected, presumably due to assumptions used in the model. However, strong and relevant differences were still observed between the two monkeys suggesting that our measurement is sensitive to brain energy metabolism impairment and that direct ^{17}O MRI could be a powerful technique to study abnormal oxygen consumption in neurodegenerative diseases.

REFERENCES [1] Zhu *et al.*, *NMR in Biomed* 18, 83-103 (2005) [2] Cui *et al.*, *JCBFM* 33, 1846-1849 (2013) [3] Atkinson *et al.*, *NeuroImage* 51, 723-733 (2010) [4] Brouillet *et al.*, *J Neurochem* 95, 1521-1540 (2005) [5] Chaumeil *et al.*, *Proc Natl Acad Sci USA* 106, 3988-3993 (2009) [6] Boumezbeur *et al.*, *Proc Intl Soc Mag Reson Med* (2008)

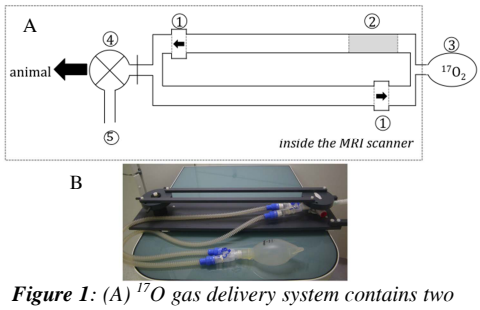

Figure 1*: (A) ^{17}O gas delivery system contains two one-way valves (1), a CO_2 absorber (2), a 1 L breathing bag (3), a three-way stopcock (4), and an in/outlet port for ventilation before/after ^{17}O inhalation (5). (B) Closed-loop breathing system and frame.*

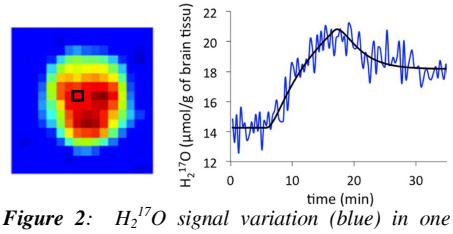

Figure 2*: $H_2{}^{17}O$ signal variation (blue) in one voxel (black square) in one axial slice and best fit (black) using the three-phase model [3]: $CMRO_2$=1.07 µmole/g of brain tissue/min, K_G=0.18 and K_L=0.33.*

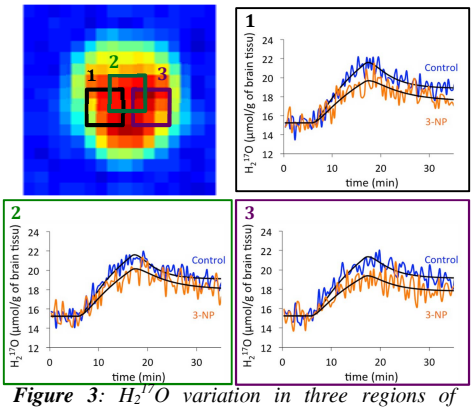

Figure 3*: $H_2{}^{17}O$ variation in three regions of interest in the control (blue) and 3-NP (orange) monkey brain.*

		CMRO$_2$	K$_G$	K$_L$
Control	**1**	0.88	0.12	0.24
	2	0.95	0.16	0.29
	3	0.82	0.14	0.26
3-NP	**1**	0.52	0.05	0.15
	2	0.57	0.06	0.17
	3	0.54	0.08	0.23

Table 1*: CMRO$_2$ (µmole/g of brain tissue/min), K_G and K_L in three regions in control and 3-NP treated monkeys.*