# Early metabolic changes in the amyotrophic lateral sclerosis SOD1 mouse brain are revealed using $^{1}$H MRS rather than CASL and $^{18}$FDG PET

**H. Lei[1,2], E. Dirren[3], C. Poitry-Yamate[1], B. L. Schneider[3], P. Aebischer[3], and R. Gruetter[1,4]**

[1]Laboratory of Functional and Metabolic Imaging, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, [2]Radiology, Univeristy of Lausanne, Lausanne, Switzerland, [3]Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, [4]Radiology, University of Geneva, Geneva, Switzerland

**INTRODUCTION** Amyotrophic lateral sclerosis (ALS) is a debilitating and fatal neurodegenerative disease of unknown etiology affecting motor-neurons of the CNS and PNS, and for which diagnostic criteria are sorely needed. Mice overexpressing G93A mutant form of human superoxide dismutase 1 (SOD1) develop a progressive limb paralysis that closely mimics the ALS. Therefore, they represent a model of choice for elucidating the biochemical/metabolic changes that may occur in affected CNS regions. The progressive nature of this disease highlights the need for longitudinal and non-invasive measurements of brain function, structure and metabolism. We have exploited the G93A SOD1 mouse model to explore regional biochemical variations in brain and brainstem during the pre- and post-symptomatic phases using $^{1}$H MRS (1,2) CASL (3) and $^{18}$FDG PET imaging (4).

**METHODS** G93A-SOD1 mice were bred and genotyped as previously described (5). Based on preliminary electromyographical measurements and swimming abilities, we designated post-natal day 60 (P60) as pre-symptomatic, and P100 as mildly post-symptomatic. The mean lifespan of our animals was ~-125 days. During the entire experimental period, animals were maintained under isoflurane anesthesia (0.8-1.5%) and physiological parameters were continuously monitored. At 9.4T, localized $^{1}$H MRS was applied on motor cortex, striatum and brainstem using SPECIAL (2 and references therein). Water signals with no water suppression (NT=8) were acquired for further absolute quantification. Concurrently, cerebral blood flow was evaluated randomly in three animals at P60 using CASL techniques (3) with a home-built active-detuned system, including a butterfly coil (8-mm-diameter) for labeling and a quadrature coil (two geometrically decoupled 12-mm-inner-diameter loops) for imaging. 16 pairs of four-segmented semi-adiabatic SE-EPI with a negative and positive gradient scheme (1-mm thickness, FOV=23X15mm$^2$, RO/PE=128/64, TE/TR=40/3000ms, a 2-sec labeling pulse along with a 1.4G/cm z-gradient, 6) was applied and the total acquisition time was 15 minutes. The labeling efficiency was close to 0.8 (7) and cerebral blood flow maps were calculated from the images (3). MR spectra were processed and quantified using the LCModel (1, 2 and references therein). Water content was 80% for motor cortex and striatum and 75% for brainstem (preliminary data not shown). We assumed no water content changes between P60 and P100. Numerous metabolites (names and the corresponding abbreviations were as in references 1 and 2) were detected with CRLB <50%. One week after P100, mice and age-matched controls (5 per group) subsequently underwent $^{18}$FDG PET imaging of the head and neck regions following a ~45MBq $^{18}$FDG intravenous bolus. Trapped, intracellular 18FDG-6-phosphate was quantitated using Standard Uptake Values (SUV): [mean ROI activity (kBq/cm$^3$)] / [injected dose (kBq)/body weight (g)].

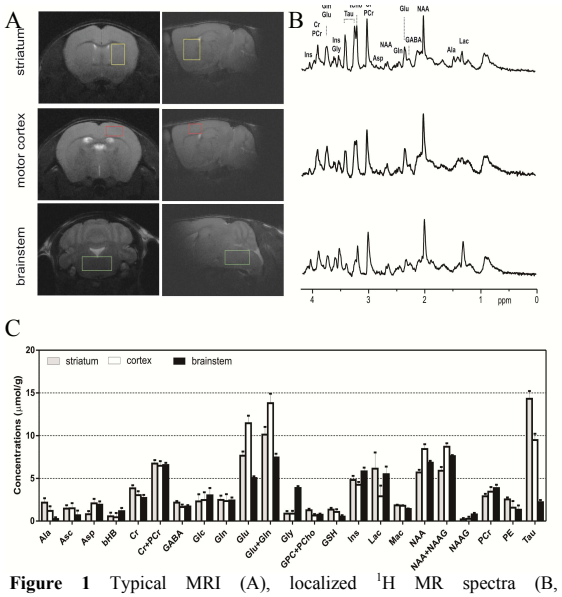


**Figure 1** Typical MRI (A), localized $^{1}$H MR spectra (B, TE/TR=2.8/4000ms) and neurochemical profiles (C) of mouse striatum (~5.6µl, NT=320), motor cortex(~6µl, NT=320), and brain stem (~13µl, NT=400-480) at 9.4T. Error bars are SDs.

| | | Relative increase (±%, *p-value*) | Relative decrease (±%, *p-value*) |
|---|---|---|---|
| P60 | ***Brainstem*** | Glu (+8, *0.017*) | Lac (-19, *0.04*) |
| | ***Motor cortex*** | Asc (+51, *0.017*) | GABA (-30, *0.003*)<br>NAA (-5, *0.04*) |
| | ***Striatum*** | Glu (+13, *0.02*) | |
| P100 | ***Brainstem*** | Gln (+33, *0.0005*)<br>Asp (+49, *0.03*)<br>PCr (+20, *0.006*) | Lac (-21, *0.04*) |
| | ***Motor cortex*** | Gln (+22, *0.02*) | Glu (-12, *0.0004*)<br>Lac (-61, *0.002*)<br>Ins (-14, *0.03*) |
| | ***Striatum*** | PCr (+22, *0.006*) | Lac (-44, *0.03*)<br>Cr (-11, *0.004*) |

**Table 1** Summary of significant metabolic changes observed at P60 and P100 (unpaired t-test).

**RESULTS AND DISCUSSION** At 9.4T, quality anatomical imaging data allowed precise localization of the volume of interests (VOIs) in SOD1 mouse brain, striatum, motor cortex and brainstem (Figure 1A). With improved field homogeneities, and satisfactory water suppression performance and sufficient scan number, localized MR spectra of striatum, motor cortex and brainstem were obtained with satisfactory quality (Figure 1B). The resulting linewidths and SNRs were 7±1Hz and 14±3 in striatum; 10±1Hz and 14±3 in motor cortex; and 13±2Hz and 16±2 in brainstem. The resulting neurochemical profile of 20 metabolites per region was quantified using the LCModel (Figure 1C). Regional metabolic profiles were quantitatively distinguishable from MR spectra (Figure 1, p<0.0001, two-way ANOVA). While nearly identical blood flows were observed in the motor cortex of G93A SOD1 and age-matched mice, i.e. 86±12 vs. 83±3 ml/100g/min respectively, metabolic changes were observed in motor cortex, striatum and brainstem of SOD1 mice as early as P60 (Table 1). At P100, the level of lactate in diseased mice was decreased in all 3 regions (Table 1), in contrast to plasma lactate levels which were comparable to that in controls. This finding is contrary to previously reported increases of lactate levels in SOD1 mice obtained *in vitro* (8), and which could be biased by postmortem effects. A general trend towards lowered $^{18}$FDG metabolism in CNS (p-value>0.05) was revealed in >P100 SOD1 mice using PET measurements. This suggested that $^{18}$FDG metabolism in all three brain regions was possibly not altered at P100. Overall, our results indicated that the progression of over-expressing superoxide dismutase in mice (Table 1) may be linked to excitotoxicity(9), i.e. increased glutamate levels at P60 and followed by increased glutamine levels at P100. In conclusion, this is first time to report *in vivo* $^{1}$H MRS studies of mouse brainstem. Whatever the underlying pathogenic mechanisms, studying such animal models longitudinally using $^{1}$H MRS is an effective and powerful strategy towards identifying potential biomarkers of ALS during the early post-symptomatic phases.

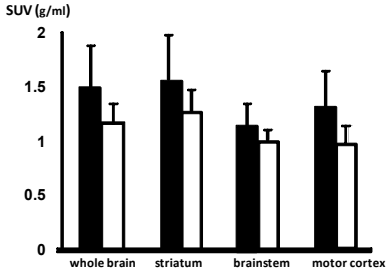


**Figure 2** Summary of $^{18}$FDG metabolism at >P100 (p-value>0.05, unpaired t-test).

**REFERENCES 1.**Tkac I, et al., Magn. Reson. Med. 2004; 52(3): 478–484; **2.**Lei H et al., J. Cereb. Blood. Flow. Metab. 2009; 29(4): 811–819; **3**. Choi et al. J. Cereb. Blood. Flow. Metab. 2001 ; 21(6) :653-663. **4**. Viertl D et al. Mol Imaging Biol 2010 ; **5**. Niessen HG et al. Eur J Neurosci 2007; 25:1669-1677 ; **6.** Van de Looij Y et al. Magn Reson Med 2010 (In Press) **7.** Muir ER et al. Magn Reson Med 2008 ; 60(3) :744-748 **8**. Guillot S et al. Neurobio Dis 2004;16:139-149 ; **9.** Van den Bosch L et al. Biochimica et Biophysica Acta 2006; 1762:1068-1082

**ACKNOWLEDGMENTS** Supported by Centre d'Imagerie BioMédicale (CIBM) of the UNIL,UNIGE, HUG, CHUV, EPFL, SNF 3100A0-122498, the ALS association, Swiss Foundation for Research on Muscular Diseases (FSRMM) , and the Leenaards and Jeantet Foundations.