# Magnetic Resonance and Detailed Histological Analysis of the Primary Motor Cortex in Amyotrophic Lateral Sclerosis

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**Introduction:** Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder primarily involving the motor neurons in the cerebral cortex, brainstem and spinal cord (1). Most cases of ALS occur sporadically and are of unknown etiology such that the progression of the cellular events in the nervous system from initial insult to final cell death is unknown. Loss of neuronal function within the primary motor cortex (PMC) is common in late stage ALS with neurons in the upper motor region having already been profoundly affected when diagnosis is made solely on clinical manifestation of the disease (2). An understanding of the etiology and resulting in ALS would aid in monitoring the advancement of the disease. Here we present data on the imaging of cadaver ALS brains and detailed histological analysis of signal changes in the primary motor cortex.

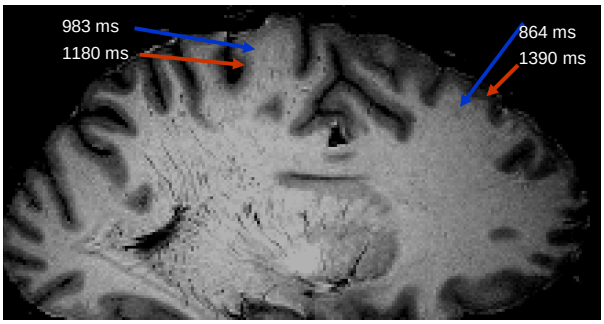


Figure 1: *Ex vivo* $T_1$ weighted image of an ALS cadaver brain. Relaxation measurements of gray matter (red arrow) and white matter (blue arrows) are given in the PMC (left) and the frontal lobe (right).

**Methods:** Seven cadaver brains from clinically determined ALS patients were excised during autopsy. The brains were bisected sagittally and placed into Alginate dental impression polymer. A $T_1$ weighted 3D image set with a 20 x 20 x 6.0 cm FOV, 256 x 256 x 60 matrix, 25.33ms TR, 4.8ms TE, 8 NEX was obtained in 140 minutes. After completion of scanning protocols, control and ALS brain tissue was fixed in 10% Low Odor Formalin for one week. Following fixation, gray matter from the primary MC (PMC), secondary MC, primary somatosensory, frontal and occipital lobes were excised, embedded in paraffin and sectioned at 15µm thick. For each region of interest (ROI), five age-matched control brain tissue regions were obtained from the Harvard Brain Bank and processed with the ALS tissue samples. Sections were stained with antibodies for neuronal filament to detect neuronal bodies and processes, glial fibrillary acidic protein (GFAP) to view astrocytes and Luxol fast blue to observe myelinated axons. Cell counts were made for GFAP positive cells in ALS and control brains. The degree of positive Luxol stained myelin was determined with densitometry measurements in all tissue types. Prior to encasing select brains in the Alginate for MR imaging, 1mm cubed sections were taken out of the RIO's for processing and transmission electron microscopy.

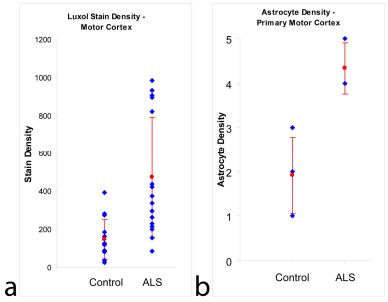


Figure 3: (a) Average Luxol stain densitometry measurements in the PMI of controls and ALS in the PMI. (b) Astrocyte cell counts in the PMI for controls and ALS tissue samples in the PMI.

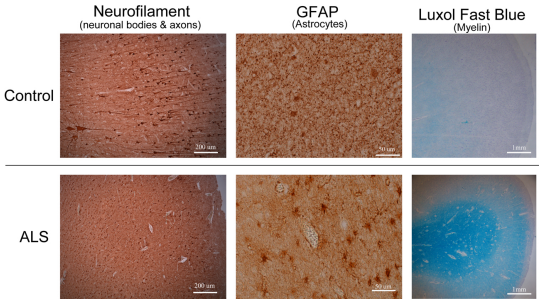


Figure 2: Neurofilament in gray matter, GFAP in white matter and Luxal Fast blue stains for control (top) and ALS (bottom) PMI tissue sections.

**Results:** Figure one shows a representative $T_1$ weighted image of an ALS cadaver brain. The $T_1$ relaxations in gray matter for the PMC shows a decrease compared to other brain regions. Conversely, $T_1$ relaxation for white matter shows an increase in the PMC. Neurofilament staining for neurons and axons, GFAP staining for astrocytes and Luxol fast blue for myelin density are seen for both controls and ALS tissue samples in figure 2. The neurofilament stains shows increased amounts cell counts of positive staining for neurons and axons in the control brains. GFAP positive astrocytes are more prevalent and reactive in the ALS brain compared to controls. Myelin staining is more intense in the ALS brain samples compared to controls. Quantification of the average densitometry measurements for the Luxol stain are seen in figure 3a. The average densitometry measurements of the controls were significantly lower ($P < 0.001$) than ALS samples at $147.5 \pm 103.5$ and $472.6 \pm 318.3$ (respectively). Average cell counts for GFAP (Fig. 3b) was significant higher in the ALS tissue ($P < 0.002$) verses controls with cell density counts of $4.33 \pm 0.57$ and $1.93 \pm 0.86$ respectively. Transmission electron microscopic images show that the myelin sheaths around the axons in the affected PMC are unwound, distressed and fewer in number compared to frontal and occipital lobes regions.

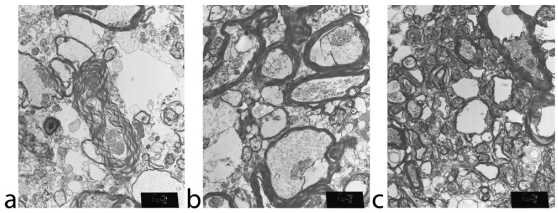


Figure 4: Transmission Electron Microscopy images taken at a magnification of x7700 of the (a) PMI, (b) frontal, and (c) occipital lobes.

**Conclusions**: MR images of freshly excised cadaver brains show a lack of gray/white matter boundary contrast within the primary motor cortex, when compared other brain regions. The overall trend is a decrease in signal and relaxation time in the gray matter and an increase in signal and $T_1$ relaxation in white matter. The various staining methods show quantifiable differences between ALS and control tissue samples. The data indicate an increased amount of gray and white matter disturbance and distress as indicated with increased amounts of reactive astrocytes, decreased counts of neuronal bodies and axons, more robust Luxol fast blue staining in the ALS tissue samples and unwinding of the myelin sheath. These results provide insight into the cause of the MR intensity and relaxation differences that are seen in the ALS cadaver brains.

**References:**

1. Hong *et al*., Journal of Neurological Sciences. 2004; (227): 73 – 78
2. Abe *et al*,. NMR in Biomedicine 2004; 17: 411 – 416.