Ex-vivo MR volumetry of human brain hemispheres.

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Target Audience: Researchers in MR neuroimaging of aging.

Purpose: Ex-vivo MRI of human brain hemispheres provides images at essentially the same time-point as histological examination of the tissue, ensuring that no additional pathology develops between imaging and histology[1]. This may allow investigation of the effects of various neuropathologies occurring in aging, on the volume of different brain regions. However, the effects of death, brain extraction from the skull and chemical fixation on brain hemisphere volumes are largely unknown. Thus, the value of ex-vivo brain MR volumetry remains uncertain. The purpose of this study was two-fold: 1) longitudinally assess the volume of various gray matter regions measured with ex-vivo MRI, and 2) investigate the relationship between volumetric measurements performed in-vivo and ex-vivo.

Methods: MRI Data: All human subjects were recruited from a longitudinal clinical-pathologic study of aging, provided written informed consent, and signed an anatomical gift act. Brain hemispheres were immersed in formaldehyde solution immediately after extraction from the skull (48 ± 27 days), and were imaged while immersed in formaldehyde solution[2]. (*Dataset 1*) Cerebral hemispheres from five elderly subjects (age-at-death = 83.9 ± 6.7 years) were imaged ex-vivo on a weekly basis for three months with an additional scan at six months postmortem. (*Dataset 2*) Cerebral hemispheres from seven elderly subjects were scanned both in-vivo and ex-vivo (age-at-death = 88.7 ± 1.7 years). The in-vivo acquisitions were performed less than 2 years before death. All ex-vivo imaging was conducted at 3 T.

Automated Segmentation: Forty-two cortical and sub-cortical gray matter regions were segmented in all hemispheres imaged ex-vivo using multi-atlas segmentation[3]. The atlases used for multi-atlas segmentation consisted of in-vivo high-resolution anatomical data from 25 subjects, previously segmented using FreeSurfer (http://surfer.nmr.mgh.harvard.edu) and divided into individual cerebral hemisphere volumes. All atlases were registered to each ex-vivo dataset, and all labels were transferred to each hemisphere's space. The final segmentation was obtained using a vote-rule based on maximum frequency of appearance of a label. The results were further improved using tissue maps obtained using FAST (FSL, Oxford, UK), and manual correction of gray/white matter tissue boundaries.

Longitudinal Assessment of Gray Matter Volumes Measured with Ex-vivo MRI: The volumes, V(r,t), of gray matter structures, r, measured in *Dataset 1* at different time-points, t, were plotted as a function of the volumes measured at the first time-point, V(r,1). Linear regression was performed to test for significant linear relationships between volumes measured at the first and all other time-points ($P<0.05$). Also, the volumes of gray matter regions measured at each time-point were normalized by the volumes of the same regions measured at the first time-point, $V'(r,t) = V(r,t) / V(r,1)$. The mean of $V'(r,t)$ over the whole hemisphere was plotted as a function of time postmortem. Linear regression was used to detect significant trends over time postmortem ($P<0.05$).

Relationship Between MR Volumetric Measurements Performed In-Vivo and Ex-Vivo: The ex-vivo volumes of each subject of *Dataset 2* were plotted as a function of the corresponding in-vivo volumes. Linear regression was used to test for significant association between the volumes of brain regions measured ex-vivo and in-vivo ($P<0.05$).

Results and Discussion: For all hemispheres of *Dataset 1* imaged at multiple time-points postmortem, linear regression revealed statistically significant linear relationships (slope≈1, $R^2>0.9$, $P<10^{-20}$) between the volumes of all regions measured at a certain time-point, V(r,t), and volumes of the same regions measured at the first time-point, V(r,1), for all time-points, t (Fig.1A). Furthermore, linear regression of the mean of $V'(r,t)$, showed no significant trend over time postmortem for the first 6 months after death, for any hemisphere of *Dataset 1* (Fig.1B). These results suggest that, from approximately one week postmortem to 6 months postmortem, the volumes of individual gray matter regions remain unchanged, as long as hemispheres remain immersed in formaldehyde solution[2]. This information will be crucial for future cross-sectional studies involving ex-vivo MR volumetry of human brain hemispheres imaged at different postmortem intervals.

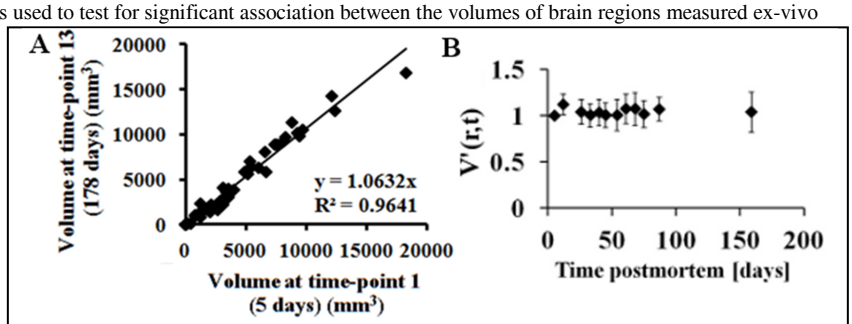

Figure 1: A) Plot of the volumes of different gray matter structures at the 13th time-point vs. the corresponding volumes at the 1st time-point. B) Plot of the mean $V'(r,t)$ over the whole hemisphere vs. time postmortem. These plots present representative results from a single hemisphere. Similar plots were obtained for all time-points (A) and all hemispheres (A, B).

For each subject of *Dataset 2*, linear regression showed a statistically significant linear relationship ($P<10^{-20}$) between the volumes of brain regions measured ex-vivo and in-vivo (Fig. 2). The slope of these linear relationships was higher than one, suggesting that the volumes measured ex-vivo were larger than those measured in-vivo. However, differences in image quality between the in-vivo and ex-vivo MRI data, as well as the different segmentation methods used, introduced a certain weighting in the association between volumes measured in-vivo and ex-vivo. Consequently no conclusions can be drawn from the slope of the linear relationship between MR volumetric measurements performed ex-vivo and in-vivo. Nevertheless, the linear behavior itself suggests that the presented approach for ex-vivo MR volumetry captures information that is linked to the ante-mortem macrostructural characteristics of the brain (an important first step for translation).

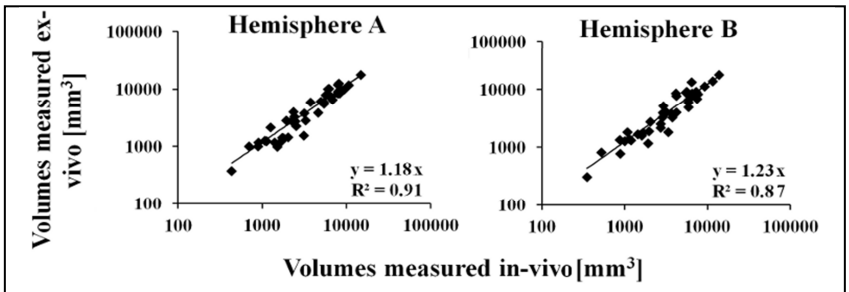

Figure 2: Plots of the volume of gray matter structures measured ex-vivo versus the corresponding volumes measured in-vivo (results from 2 of the 7 subjects of *Dataset 2*).

Conclusion: This work demonstrated that brain gray matter volumes measured ex-vivo remain unchanged for a period of 6 months postmortem, and are linearly related to the volumes measured in-vivo on the same subjects. Ex-vivo MRI ensures that no additional pathology develops between imaging and histology, and accomplishes that in a cost effective manner in contrast to the alternative of longitudinal in-vivo MRI. Furthermore, ex-vivo MRI allows higher image quality than in-vivo MRI, leading to more accurate volume measurements. Finally, frail elderly subjects, an important population for aging research, can only be imaged ex-vivo. Thus, based on the above, combination of ex-vivo MR volumetry and histopathology may become an effective tool for the assessment of the neuropathologic correlates of macrostructural brain abnormalities.

References: [1] Pfefferbaum A, et al., NeuroImage 2004;21:1585. [2] Dawe RJ, et al., PLoS One 2011;6:e26286. [3] Heckemann RA, et al., NeuroImage 2006;33:115.