Paraformaldehyde and glutaraldehyde fixations preserve manganese enhancement in ex vivo mouse brain MRI

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Introduction. Manganese enhanced MRI (MEMRI) for *in vivo* bioimaging is limited in spatial resolution and signal sensitivity. This is due, in part, to limited scan times[1]. Importantly, *ex vivo* brain MRI can achieve superior resolution and signal-to-noise ratio through increased signal averaging and the use of solenoid RF coils optimized to brain size. Maintenance *of in vivo* manganese enhancement for *ex vivo* MRI was developed using different fixation methods including cross-linking chemicals and focused beam microwave irradiation (FBMI).

Methods and Materials. Twelve mice (NOD/scid) were administrated $MnCl_2$ through tail veins then scanned by MRI 24 hours later (fast spin echo with variable TR for T_1 mapping, and fast spin echo with TR = 300 ms for T_1-wt). The animals were then killed and the mouse brains soaked in the s4% paraformaldehyde (PFA) + 0.1% glutaraldehyde (GA) (Fixative I, *n* = 3), 1.5% GA (Fixative II, *n* = 3), or 3% GA (Fixative III, *n* = 3). FBMI was used on each of the remaining three mice. Fixed brains were scanned again *ex vivo* to measure relaxation times and to acquire MR images. Another group of three mice was scanned for baseline measurements, and fixed without $MnCl_2$ infusion. Fixed brains were then harvested and soaked in a 0.24 mM $MnCl_2$ solution [2]. The tissue relaxation times of these brains were measured after seven days, and T1-wt MRI were acquired using fast spin echo with TR = 60 ms. All T_1-wt MRI was acquired with 3D isotropic 100 μm resolution.

Results. *T_1 values and T_1-wt signal on major neurostructures* Mn^{2+} generated significant T_1 reduction ($p < 0.05$) in *in vivo* MRI scans (Table 1). All chemical fixations did not demonstrate any significantly change in T_1 compared to Mn^{2+} enhanced (ME) *in vivo* measurements. FBMI fixed animals showed reduced T_1 values. No significant differences were found between the structures in FBMI mice. (Table 1, Figure 1). Significant T_1 reductions on basal ganglia (BG), thalamus (TH) and midbrain (MB) compared to cortex (CTX) on mice fixed using Fixative I and II are shown in Tab 1 ("[b]": $p < 0.05$). In Fig 1, similar relative signal intensity patterns could be observed on ME *in vivo* and ex vivo MRI of chemically fixed animals (only fixative I is shown in Fig 1): higher signal intensity on BG, TH and MB than on CTX; and similar intensity on BG, TH and MB. In FBMI fixed mice, T_1-wt signal contrast has been lost. The brains soaked in $MnCl_2$ showed higher T1-wt intensity on CTX compared to BG, TH and MB.

Table 1. T_1 values on major neurostructures

	CTX	BG	TH	MB
Baseline	1742 ± 62	1679 ± 43	1599 ± 57	1571 ± 47
ME In vivo	1123 ± 29	997 ± 30[b]	989 ± 43[b]	961 ± 44[b]
Fixative I	1086 ± 55	916 ± 94[b]	931 ± 16[b]	808 ± 85[b]
Fixative II	1080 ± 6	904 ± 42[b]	896 ± 27[b]	982 ± 33[b]
Fixative III	1125 ± 59	1002 ± 25[b]	1028 ± 56[b]	1118 ± 29
FBMI	954 ± 55[a]	892 ± 54	859 ± 17	817 ± 4

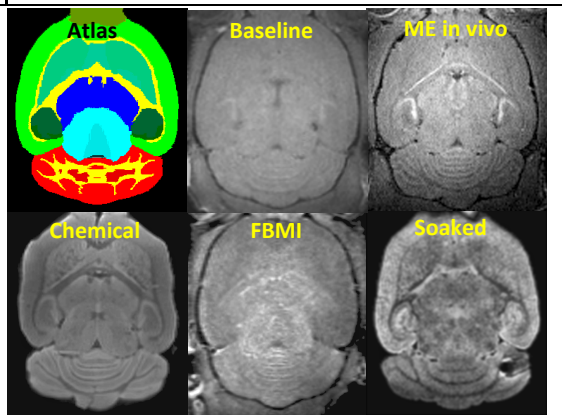

Figure 1. Axial slices of T_1-wt MRI showing CTX, BG, TH and MB

T_1 values and T_1-wt signal on sub-structures Manganese induced great T_1 reduction on several sub-structures in *in vivo* MRI (Tab 2). In all ME *in vivo* and *ex vivo* measurements on mice fixed using chemicals, T_1 values were significantly lower on the granular layer of dentate gyrus (GrDG) and the pyramidal layer (Py) compared to CA regions. Similarly, the cerebellar cortex (CBX) granule cell layer (GrCBX) has lower T_1 than other layers of CBX ("CBX other" in Tab 2). The signal enhancement on Py and GrDG is preserved on ex vivo MRI (Fig 2). The 10 cerebellum lobules can be clearly identified on in vivo and ex vivo T1-wt MRI of chemically fixed brains (Fig 3). The granular layer of CBX obtained higher enhancement compared to others including the molecular cell and Purkinji cell layers (Fig 3). In the soaked brains, GrDG and Py were enhanced less compared to CA regions, and the granular layer of CBX was not enhanced compared others.

Discussion The results of T_1 measurements and T_1-wt MRI indicated that in vivo localization of Mn^{2+}, the binding of Mn^{2+} to macromolecular structures, and the longitudinal relaxation mechanism of Mn-macromolecule compounds in brain tissue were not significantly affected by the chemical fixatives. It has been proven that microwave can preserve metabolites in the brain for a considerable period of time. The loss of signal contrast among neurostructures suggests that microwave heating causes opening of the ion channels in neurons, causing dispersion of Mn^{2+}. The measurements on brains soaked in $MnCl_2$ generated similar results in a previous study[2]. Contrary to *ex vivo* MRI of chemically fixed brains, the soaked brains showed higher T_1-wt signal intensity on CTX than BG and TH. One reason could be that the diffusion of Mn ions was impeded. The other reason may be that cortex has more binding sites for Mn ions than basal ganglia and thalamus. Nevertheless this study has shown that cross-linking fixations preserve the Mn^{2+} enhancement.

References 1.Akoi et al., Neuroimage. 2004 Jul;22(3):1046-59., 2. Huang et al. Neuroimage. 2009 Jul 1;46(3):589-99

Table 2. T_1 values on sub-structures

	GrDG/Py	HIP-CA	GrCBX	CBX other
Baseline	1668 ± 22	1798 ± 67	1634 ± 52	1729 ± 54
ME In vivo	809 ± 42	1269 ± 44	893 ± 36	1212 ± 61
Fixative I	943 ± 69	1186 ± 80	839 ± 38	1145 ± 38
Fixative II	854 ± 32	1162 ± 36	945 ± 9	1179 ± 41
Fixative III	1015 ± 6	1274 ± 63	1027 ± 28	1283 ± 63

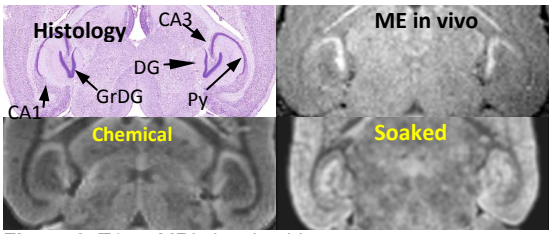

Figure 2. T1-wt MRI showing hippocampus

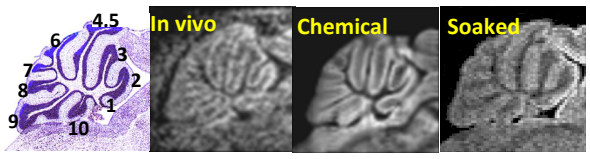

Figure 3. Sagittal slices of T1-wt MRI showing cerebellum