Towards High Throughput *In Vivio*</I> Brain Phenotyping in Mice

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Introduction: MRI is adept at imaging soft tissue *in vivo*, which makes it an ideal modality for visualizing mouse neuroanatomy. One particular application of MRI is mouse brain phenotyping, in which MRI is used to screen mice with known behavioural defects for abnormalities in brain structure. The purpose of this project is to test a high throughput *in vivo* MRI screen to detect a known mutation in mice that are homozygous for the cerebellar deficient folia (*cdf*) mutation, which causes abnormal morphology in the cerebellum and hippocampus[1]. To characterize this subtle mutation, we measured the volumes of the whole brain, the cerebellum, the hippocampus, and the ventricular system in a population of wild-type mice and compared those to the same measurements in *cdf/cdf* mutant mice.

Imaging Methods: Five wild-type mice and three *cdf/cdf* mice bred in the inbred C3H/HeSnJ background (The Jackson Laboratory, Bar Harbor, Maine) were imaged on a 7-Tesla MRI scanner (Varian, Palo Alto, California) outfitted for seven multiple-mouse MRI[2]. The mice were injected with 20 mg/kg $MnCl_2$ and imaged 48 hours later[3] with a 3D spin-echo pulse sequence in 2 hours and 45 minutes (TR=300 ms, TE=10 ms, readout x phase x phase = 256 x 128 x 128 = 4 cm x 2 cm x 2 cm, NEX=2) to produce $(156\ um)^3$ isotropic imaging voxels. The mice were anaesthetized with 1% isofluorane in O_2 during imaging and their body temperatures were maintained at 37° C with flowing hot air.

Image Analysis: To facilitate labeling, the 3D MR image of each mouse's brain was nonlinearly registered into an unbiased, common co-ordinate space[4]. In the resulting average atlas image, brain structures of interest (whole brain, cerebellum, hippocampus, and ventricles) were drawn in each slice and labeled using AMIRA software (TGS, San Diego, California) The labels were then deformed back onto each mouse's original image using the inverse of the deformation fields generated by the registration. The automated labeling was confirmed by eye. This saved us the lengthy step of manually labeling each mouse's brain. Figure 1 shows the resulting labeling in a wildtype mouse (Left) and a cdf mutant mouse (Right), with the cerebellum in blue, the hippocampus in orange, and the ventricles in green.

Results and Discussion: The volumes of the labels from the native brains are shown in Table 1. The standard deviations of the volume measurements in the wild-type mice are small and we can detect volume changes in the cerebellum, lateral ventricles, and third ventricles in the mutant mice at the p=0.01 level (denoted by * in Table 1). As shown before from histology[1], a defect in the folia of the cerebellum (MRI shown in Figure 2) causes the cerebellar volume change in the mutant mice. The technique has the advantage of being almost completely automated and we are currently investigating metrics other than simple volume measurements to better characterize the abnormal neuroanatomy in the mutant mice. Ultimately, our technique can be used to detect abnormal brain phenotypes in novel mutant mice.

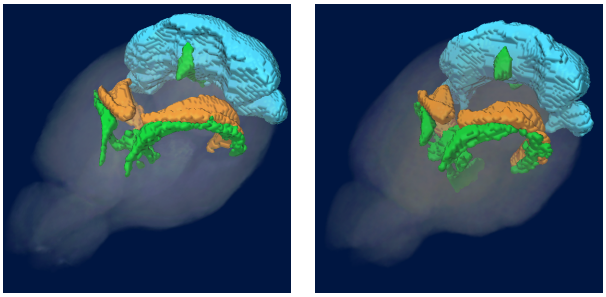

Figure1. Left Wildtype. **Right** Mutant.

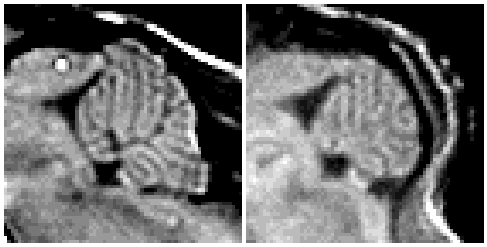

Figure 2. Left Wildtype. **Right** Mutant.

Structure	Wild-type volume (mean ± std. dev. in mm^3)	*cdf/cdf* volume
Whole brain	482 ± 30	465 ± 20
Cerebellum *	63 ± 1	45 ± 5
Hippocampus	6.2 ± 0.3	5.3 ± 1
Fourth ventricle	0.68 ± 0.06	0.66 ± 0.06
Third ventricle *	0.51 ± 0.05	1.55 ± 0.2
Lateral ventricles *	1.61 ± 0.08	2.22 ± 0.2

Table 1.

References:

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