INTEGRATED FUNCTIONAL GENOMICS OF THE MOUSE
CONFERENCE 6 and 7 OCTOBER 2003

ABSTRACT

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SUMMARY

Lay summary (around 50 words)

The Mouse Imaging Centre is developing high throughput imaging screens using magnetic resonance imaging (MRI), ultrasound biomicroscopy and micro-computed tomography for the examination of ENU-mutagenesis derived mice and for brain cancer models. We show preliminary results from an MRI brain atlas, a secondary screen of cardiac mutants and *in vivo* screens of mice with brain cancer.

SCIENTIFIC ABSTRACT

Detailed scientific description of your research interests of relevance to EUMORPHIA (up to 2 sides of A4 paper)

CURRENT PROGRESS OF IMAGING SCREENS AT THE MOUSE IMAGING CENTRE

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The Mouse Imaging Centre is dedicated to creating high-throughput imaging screens using state-of-the-art radiological technologies adapted for mice. The need for such techniques is growing as the functional genomics agenda turns towards mice. Specifically, we work with the Centre for Models of Human Disease in Toronto, Canada which generates randomly mutated mice. In addition, there are many additional targeted mutagenesis programmes within the University of Toronto research community.

Excised mouse brain atlas

The success of screening strategies is fundamentally dependent on the determination of normal versus mutant. For a "one-dimensional screen", such as blood pressure or ECG, the discrimination is relatively simple: take a large control population, average the measured parameters and estimate the variance. In imaging, however, the parameter space is astronomically larger; an interpretation of an parameter could range from something as simple as the average total volume of the heart or as complicated as the average shape distribution of the kidneys across its modes of symmetry.

As a starting point, we have created an unbiased atlas from excised brains imaged with MRI. The brains of age-matched and sex-matched C57/BL6 (n = 10) and CD1 mice (n = 5, data not shown) were perfusion-fixed in formaldehyde, excised and imaged using a 3D spin echo sequence. For each strain, we created a 3D variational atlas which consists of an unbiased average image and n deformation fields describing how individual samples are "morphed" to the average. Construction of the variational atlas is a fully automatic, iterative
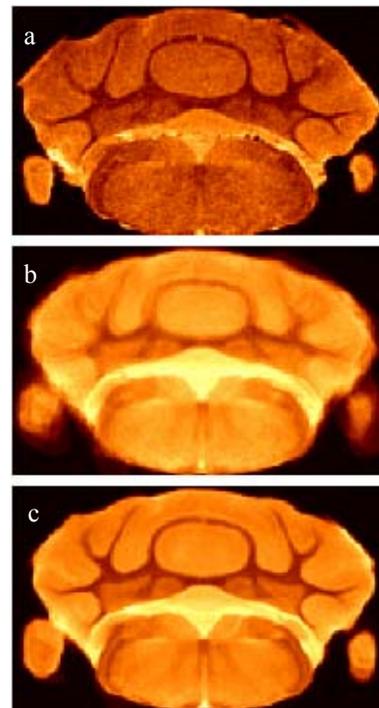
Figure 1. A coronal slice through the cerebellum from the C57/BL6 dataset: **(a)** a single brain image, **(b)** an initial low-order 9-parameter average image and **(c)** the final non-linear average image. Note the fine anatomical resolution in the atlas (c) as compared to the single brain image (a).

process involving registration, resampling, intensity normalization and averaging. Figure 1 shows a coronal slice from the C57/BL6 strain (see caption for details).

The average image produced by the process is used to define a standard reference coordinate space. The *n* deformation fields are used to measure the spatial distribution of homologous anatomical locations across all images, with respect to the reference space. We have found that both strains exhibit very low variability (around 80 μm) in most of the central brain regions. The variability is higher (up to 500 μm) along the outer brain surface and is attributed to specimen handlling.

This work introduces a method for creating unbiased, strain-specific variational atlases and formalizes the notion of normal intrastrain morphological variability. Future applications include interstrain comparisons and detection of "morphological outliers" in the living brain and the whole body.

ENU cardiovascular mutants

As a prelude to their use as a primary ENU screen, we are honing our imaging tools in secondary screens for interesting ENU mutants. For example, two G3 mice were found to have high aortic peak velocity — on average 200 cm/s as compared to 100 cm/s in controls. Subsequent ultrasound examinations indicating ventricular dysfunction were inconclusive since the age-matched controls at 60 weeks exhibited similar problems. After perfusion fixing the mice with Gd-DTPA, MRI revealed severe valvular hyperplasia in both mice and left ventricular hyperplasia in one mutant (Fig 2a) and an aortic dissection in the other (Fig 2b) as indicated by the arrows.
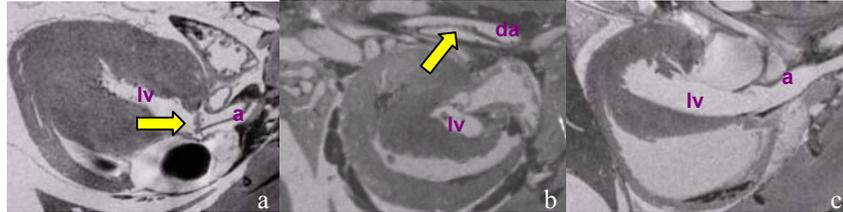

Figure 2. Single MRI slices through the long axis of the heart. **(a-b)** The two G3 mutants showed anatomical problems and **(c)** the control had normal ventricle and aortic valves.

In vivo screen for a brain cancer model

The real power of imaging is the ability to conduct time-course studies in the same mice. Because the imaging times of MRI can be long (on the order of three hours for a 3D image), we have parallelized the acquisition so that at present we can study four mice at a time. We have tested this methodology by following a cohort of mice injected with U-87 MG human astrocytoma cells. Screening started two weeks post injection and continued every week until the mice failed to thrive and were sacrificed or died (between 4 and 6 weeks). Figure 3 shows the advantage in using a 3D MRI image — the complex extent of the tumours would be impossible to characterize in a single MRI slice and would be misrepresented if multiple slices were used that were not contiguous.

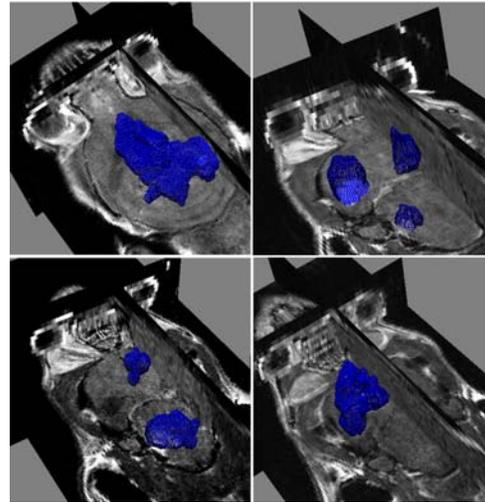Future work will be increasing the parallelization to 7 and then 16 mice.

Figure 3. Volume-rendered regions of enhancement on 3D T1-weighted images of four tumour-bearing mice imaged together at the same time point five weeks post-injection.

PUBLISHED PAPERS

References for 3 recently published full papers of relevance to EUMORPHIA

Bock, NA, Konyer, N, & Henkelman, RM. Multiple-mouse MRI. *Magn Reson. Med.* **49,** 158-167 (2003).

Foster, FS, Zhang, MY, Zhou, YQ, Liu, G, Mehi, J, Cherin, E, Harasiewicz, KA, Starkoski, BG, Zan, L, Knapik, DA, & Adamson, SL. A new ultrasound instrument for in vivo microimaging of mice. *Ultrasound Med. Biol.* **28,** 1165-1172 (2002).

Zhou, YQ, Foster, FS, Parkes, R, Adamson, SL. Developmental changes in left and right ventricular diastolic filling patterns in mice. *Am J Physiol Heart Circ Physiol.* (e-pub) 2003.