Automated morphometry of transgenic mouse brains in MR images

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CHAPTER 1

Introduction

1.1 Transgenic mouse models

Mice are thankfully exploited to study biological processes that cannot be tested in a petri dish and need to be studied *in vivo* in a real organism. Mice are not only used because they are small, easy to handle, have a fast reproduction rate and are widely available, but because mice and humans share about 97.5% of their DNA [1]. The latter statement implies that many diseases in mice and humans have a similar form of progression and show similar effects. Therefore, studying biological processes in mice will give insight on human biological processes. A mouse is called 'transgenic' if its genetic material has been altered, for instance by the introduction of human genes into its genotype. The exploitation of transgenic mice for research of human diseases is a world-wide debate, even though parallel to the development of transgenic mice, ethical committees were set up everywhere to guarantee that all transgenic animal research is performed under strict guidelines for health and wellbeing of the mice.

1.1.1 History

The first notice of mice appearing in the laboratory setting was around 1897, when the Biologist William Haacke described the effect of heritage of the coat in albino mice. Unfortunately his work is often overlooked because of his failure to supply data [2]. Therefore, the first description of genetical heritage of the color coat in mice is generally considered to be the work of the Frenchmen Lucien Cuenot, who described recessive and dominant alleles. In 1909, Clarence Cook Little developed the first inbred mouse strain to study their genotype in the hope that, one day, this

would support research on human diseases, such as cancer. Later this mouse developed into the C57BL mouse, which is now the most widely described and used genotype. The first genetically modified mice were reported in 1974 by Jaenisch and Mintz [3]. They injected mouse embryos (blastocysts) with the Simian virus 40 (SV40) DNA, a polyomavirus that has to potential to cause tumors, and showed that the mice and their offspring had inherited the SV40 DNA in their DNA. But it was not until 1982 when Brinster and Palmiter incorporated the human growth gene to a mouse model [4], that the clinical world could see the enormous advantages of exploiting transgenic mouse models for human genetic studies.

Since the early 80s, the development of standardized mouse strains (groups of mice with identical genotype) for biomedical research has expanded tremendously. Currently mouse models are available for a whole range of human diseases. Also, standardized mouse atlases were created which contain a complete set of images that have a full description of the anatomy visualized. This information is worldwide used as reference, for validation purposes and as guideline for the interpretation of one's own findings. Digital mouse atlases are currently available for the brains [5–11], the limbs [12] and even the whole body [13, 14].

1.1.2 Generation of transgenic mice

There are several ways to modify the genotype of mice [15, 16]. Here the two most common techniques are shortly explained:

Microinjection

The female reproductive cells, the oocytes are harvested mixed with sperm from the male and in a petri dish. After one spermatozoon has entered the oocyte, it takes a few hours before the pronuclei of the two cells fuse and become a so-called zygote. In that period, the linear DNA sequences of the foreign genes are typically injected by microinjection into the male pronucleus [17]. For a microinjection, special needles and cell-holders are used which are roughly 0.5 - 5 μm in diameter (see figure 1.1). After the microinjection, the oocytes are placed into the uterus of a pseudopregnant female mouse. If the integration of the gene with the DNA was successful, the offspring will express the new gene.

Injections of embryonic stem cells

A blastocyst is the very first stage of the embryo, consisting of a group of cells that will later form the embryo (embryoplast) surrounded by an outer layer of cells that will become the placenta (trophoblast). Cells that are taken out from the embryoplast are called stem cells and have to capability to develop themselves into almost any type of tissue. The DNA of these cells can be modified with high precision and will, depending on the technique, result in knock-out, knock-in or conditional mice [18]. After modification of the gene, the genetically modified embryonic stem cells are placed back in the embryoplast by microinjection. Now, the cells will behave exactly as the other cells in the embryo, resulting in a chimera mouse (figure 1.1), where the phenotype expresses a mixture of the modified genotype and the normal genotype, depending on which cells were descendants of the modified stem cells and which were

not. If the embryonic stem cells have contributed to the germ cells of the chimeras, then their offspring will all express the gene. The chimera mouse is mated with a mouse with the normal genotype, a so-called wildtype, half of their offspring will be heterozygous for the modified gene and the rest are wildtype mice. The offspring of the heterozygous mice results in mice that are either wildtypes, heterozygous for the modified gene, or homozygous for the mutated gene. The mice in the latter group all express the gene and will pass it on to their offspring, allowing a reliable production of genetically modified mice.

1.2 Mouse brain anatomy

The mouse brain is considered a valid model for human brain diseases [19, 20], since all brain structures that occur in the human brain are also present in the mouse brain and they are similarly connected to each other, although differently organized and in different volume proportions. Especially human neurophysiology and neuropathology can be well studied in mouse models [21, 22]. Human psychiatric disorders are less commonly studied as the cerebral cortex of the mice is not as highly developed as in humans [23]. Figure 1.2 displays a comparison between the human brain and the mouse brain, where a volume rendering of the whole brain (A) shows the lack of cortical folding in mice and a slice through both brains (B) shows a few corresponding brain structures.

1.3 High resolution magnetic resonance imaging

Due to the increasing amount of applications for transgenic mice, small animal devices are being developed that are capable of imaging at high resolutions ($\sim$ 10-50 μm). For magnetic resonance imaging (MRI), this resulted in scanners with a high magnetic field, from 7 T, 9.4 T up to 17 T. MRI is a highly suitably imaging modality for brain imaging, as it is not based on ionizing radiation and therefore not damaging for the subject and it can acquire the images *in vivo* as well as *ex vivo*. Furthermore, MRI allows the acquisition of functional and anatomical scans with a wide range of imaging protocols all giving different information of the brain. The most commonly used imaging protocols for mouse brain imaging are:

T_1-weighted imaging
This protocol with long repetition times (TR) and short echo times (TE) has low contrast between gray and white matter, and is typically used with contrast agent, for example to visualize the vessel structure.

T_2-weighted imaging
This protocol has short repetition times (TR) and long echo times (TE), resulting in a relatively high contrast between gray and white matter and is therefore excellent for anatomical imaging of the brain.

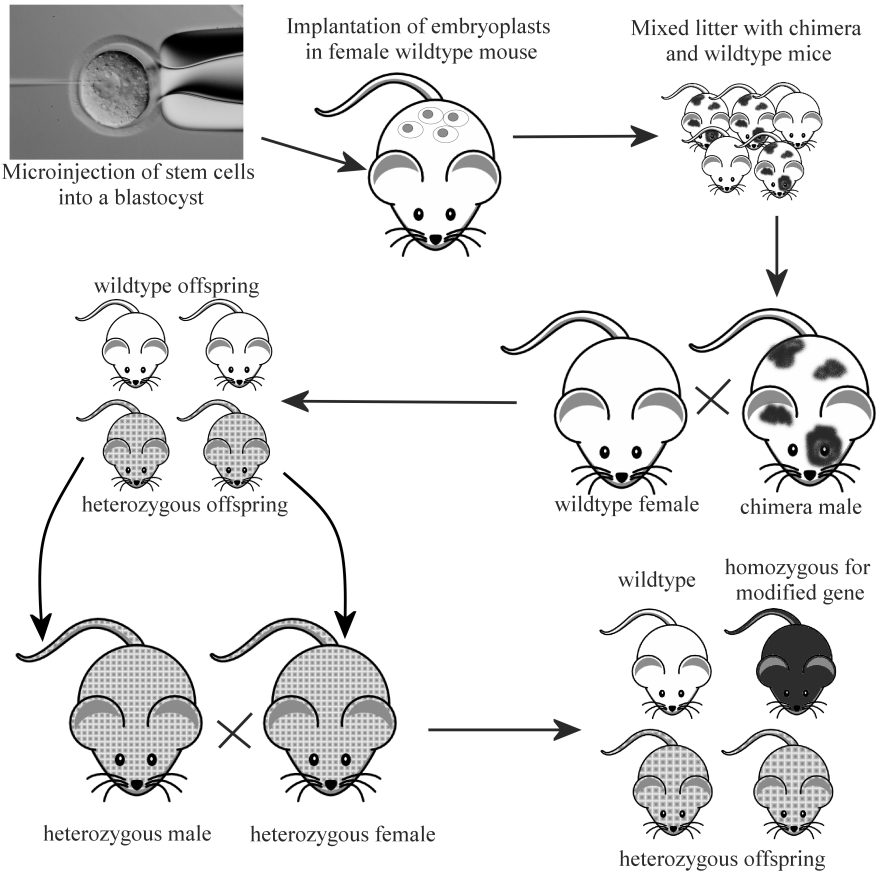

Figure 1.1: The process to generating transgenic modified mice through the implantation of embryonic stem cells, see section 1.1.2 for further details. Photography courtesy of Anne Bower and Manfred Baetscher, Transgenic Core, Oregon Health & Science University, Portland, OR. Printed with permission.

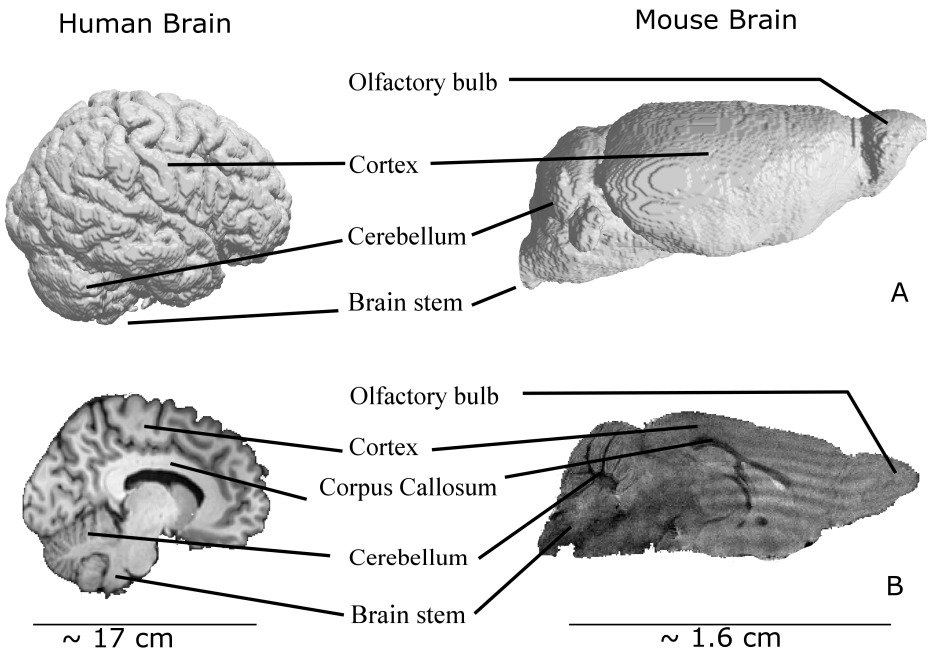

Figure 1.2: Comparison between the mouse brain and the human brain A) the outer surface and B) the internal anatomy.

Blood-oxygen-level dependence (BOLD) imaging
Protocol for BOLD imaging is sensitive for changes in the concentration of oxygenated hemoglobin, which will occur if a certain part of the brain has higher activity and requests an increased blood supply. Therefore, this technique is very suitable for functional imaging.

Diffusion tensor imaging
This protocol measures the diffusion of water in tissue. In brain tracts, the diffusion of water usually follows the direction of the tract and therefore, with diffusion tensor imaging the direction of the brain tracts can be visualized in high detail.

1.4 Aim of the thesis

To study the diseased brain, it is important to quantify local changes in the brain that occur as a result of the disease. The study of global or local shape variations in the brain is also defined as brain morphometry. In human brain MRI, automation of the morphometry process has already guided researchers to new insights regarding the (diseased) brain. With the development of MR systems for animal models, it is

now possible to acquire detailed anatomical *in vivo* images that offer the possibility to perform *in vivo* morphometry on the rodent brain. However, extending automated methods developed for human MRI to mouse brain MRI is not as trivial as it seems.

This thesis explores the possibilities for automated morphometry on MR images of mouse brains. The main contributions of this work are (a) to investigate the methods currently applied for quantitative morphometry in mouse brain MR images and (b) to provide analytical tools that can be used for the automated quantitative morphometry of mouse brains. Each chapter in this thesis is self-contained and therefore, some overlap between the chapters occurs.

1.5 Thesis outline

First, the topic of morphometry is introduced in **chapter 2** and an overview is given of the various morphometry methods and trends that are available for mouse brain MRI analysis. Furthermore, it is discussed which method is the most suitable for which situation and what the limitations and attention points are for automated morphometry. **Chapter 3** describes an application of automated morphometry on Alzheimer's Disease (AD) at an early stage in transgenic mouse brain MRI, it reviews (automated) methods in the literature. The most common transgenic mouse strains for AD are introduced, the several MR imaging parameters to detect plaques in the brain are discussed and an overview of the currently available automated methods capable of detecting AD is given.

As volumetry is the first step in quantitative morphometry, **chapter 4** presents an automated segmentation method for *in vivo* and *ex vivo* MRI, based on a hybrid method of affine registration and clustering. This clustering method is compared to manual segmentation and segmentation by nonlinear registration to evaluate its performance.

Automated morphometry is continued in the direction of deformation-based morphometry. **Chapter 5** presents the generalized Moore-Rayleigh test that tests high-dimensional vector fields for spherical symmetry and shows on simulated data how this nonparametric test can be applied to detect local brain differences between groups of transgenic mice. In **chapter 6** the Moore-Rayleigh test is further explored on experimental data of AD transgenic mice. Using synthetical and clinical data we show that the performance of the Moore-Rayleigh test outperforms the classical permutation test and significantly lowers the computational time as it is not dependent on the randomization of the data. In **chapter 7**, a clinical application of the Moore-Rayleigh test is shown in parallel with a volumetry study to phenotype a transgenic mouse model that exhibits migraine.

Chapter 8 and **9** conclude this thesis with a summary and indications for further research in English and Dutch, respectively.