ATLAS METHODS

The following paragraphs describe the methods that were used on the different version(s) of the Mouse Atlas.

MAP 2003 Atlas

In vivo MRI
Twelve week-old male C57BL/6 mice (Jackson Laboratories) were initially anesthetized with ketamine/xylzaine and then maintained on isofluorane for the duration of the imaging experiment. Diffusion-weighted uMRI images were acquired over several hours in a high-field magnet. Diffusion-weighted volumes show a great deal of anatomical detail and good contrast between gray and white matter.

Ex vivo MRI
Twelve week-old male C57BL/6 mice were sacrificed by an overdose of halothane (Sigma) according to procedures approved by the UCLA Animal Research Committee. The animals were intracardially perfused using a Minipuls II peristaltic pump (Gilson) at very low pressure with chilled PBS for approximately two minutes and FormaldeFresh (Fisher) for 15 minutes. The animals were decapitated, soft tissue removed, and the skulls were post-fixed in FormaldeFresh for 16 hours and then scanned.

Blockface and Histology
The brains were removed from post-fixed skulls and further post-fixed in FormaldeFresh for 16 hours. After post-fixation the brains were dipped in a mixture of india ink (Pelikan) and 5% gelatin (Sigma) to simplify segmentation of tissue from background later. The brains were cryoprotected in a solution of 20% sucrose for 16 hours to prevent freezing artifacts. The brains were then embedded in OCT compound (Sakura) at 4° C and snap-frozen at -70° C in a 2-methylbutane/ dry ice bath. Blockface imaging is a colorimetric imaging modality free of many of the spatial artifacts that affect serially stained sections: shatter, tears, bubbles, and other mechanical distortions. High-resolution color images of the blockface are acquired as it is sectioned, relying on the inherent contrast of white and gray matter to discriminate anatomical boundaries. Nissl-stained sections provide a wealth of information about cortical lamination and the topography of subcortical nuclei.

MAP 2001 Atlas

Mice:
Twelve week-old male C57BL/6 mice (Jackson Laboratories) were sacrificed by an overdose of halothane (Sigma) according to procedures approved by the UCLA Animal Research Committee.

Cryostat:
A CM3050S cryostat (Leica) was modified to include a micrometer (Heidenhain) that allowed the blockface to be returned to its original position (within 0.5um) and a camera mount for a DMCIe

digital camera (Polaroid) that would image the blockface prior to each section at a resolution of 1600x1200(approximately 10um/pixel).

Cryosection:
Brains were removed without perfusion or post-fixation and embedded in OCT compound (Sakura). The samples were cut serially in 50um thick coronal sections on a modified CM3050S cryostat (Leica) and images taken prior to each section. The images were acquired at a large aperture setting in combination with a short exposure time to minimize signal outside the plane of section.

Sample Preparation:
Mice were intracardially perfused using a Minipuls II peristaltic pump (Gilson) at very low pressure with PBS for approximately two minutes, FormaldeFresh (Fisher) for ten minutes, 10% sucrose in FormaldeFresh for ten minutes and finally 20% sucrose for ten minutes. The brain was removed and cryoprotected in a series of increasing sucrose concentration culminating in 30% sucrose (10% FormaldeFresh) overnight.

Histology:
Brains were cut serially in 50um thick coronal sections on a modified CM3050S cryostat (Leica) and images taken prior to each section. Sections 200um apart were Nissl-stained (Thionin) as described (LS) and alternating sections 200um apart were myelin-stained using a modified myelin impregnation stain (Gallyas 79).

Immunohistochemistry:
Image Processing. Stained preparations were digitized using a 1.25X macro objective and a 0.63X C-mount on an AX70 microscope (Olympus) with a DMCIe digital camera (Polaroid) at a resolution of 1600x1200 (approximately 10um/pixel). The digitized images were linearly registered to the blockface images using Automated Image Registration (RW) and non-linear warping was adjusted using a continuum mechanic warping algorithm (T&T 1998, 1999) based on the Cauchy-Navier operator of linear elasticity. Processing was done on an Onyx 200 supercomputer (SGI). The two-dimensional images were then reconstructed into a three-dimensional volume and quantitatively transformed into a defined and common coordinate system.

Anatomic Delineations:
Anatomic delineations were made on the digitized images of the Nissl stained sections using Illustrator 8.0 (Adobe) on a Macintosh computer (Apple). Delineations were verified with various paper atlases (GP).