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

#### Neonatal (PO) MRI Mouse Brain Atlas

Image acquisition for the subjects used to define the atlas

Eight mice from two sets of littermates were sacrificed hypothermally within 24 hours after birth, perfused intracardially with 10ml of phosphate-buffered saline (PBS) and then 10ml 2% paraformaldehyde (PFA). Animals were decapitated and the heads post-fixed with 2% PFA for 24hr before MR scans. Heads were soaked in 5% ProHance for 5 days then immersed in Fomblin for the MR scans. T2 weighted 3D spin-echo MRI images were acquired using an 11.7 T BrukerAvance imaging spectrometer with a micro-imaging gradient insert and 20 mm birdcage RF coil (Bruker Instruments). The following data acquisition parameters were used: TR/TE=300ms/6.8ms, 2 averages, FOV=12.8mm x 9mm x 9mm, matrix size= 256x128x128, T=288.1-K. The mice weighed between 1.4 and 1.5 g before being sacrificed. All animals were housed and treated in accordance with the UCLA Animal Research Committee guidelines.

# Anatomical labeling

Each MR brain image volume was firstly segmented into olfactory bulbs, cerebrum, diencephalon, midbrain-hindbrain and cerebellum. The basal ganglia (caudate putamen + globus pallidus), mammillary bodies, superior and inferior colliculi, substantia nigra, and white matter tracts anterior commissure (temporal limb) and fimbria of hippocampus, were then labeled digitally.

# Constructing the standard atlas space

Each image volume was registered to a common space with four steps. Initial alignment employed a 6-parameter linear rigid body transformation to fix the location of the origin (interpeduncular fossa) and oriented the brain. The secondary registration was accomplished with a 12-parameter affine transformation calculated using an automatic registration algorithm that maximizes 3D cross-correlation of the image intensity (1). The image volume that retained the best structural integrity and the best image contrast was selected as the registration target for the other seven image volumes. Regions outside the brains were masked before the automatic registration step was performed. This second registration step normalized the global scale. Histogram equalization was performed on the resulting images. The intensity average brain of these eight co-registered images was then created. Histogram equalization prevented an average image from being biased toward the brighter image, and thus represents an average space of the co-registered brains. This average brain then served as the new target for registration step three. Here, a 30-parameter nonlinear warping was used to reduce regional variations between subjects. Finally, a feature-based registration was performed to maximize the mutual information between the anatomical label volume of each subject and the label volume drawn on the intensity average volume from the eight co-registered brains. Retrieved displacement fields were then used to resample each image volume (2). A newly averaged image of these eight revised co-registered brain forms the standard atlas space.

## Neonatal (PO) MRI Mouse Brain Atlas continued

## Defining the probability maps

The anatomical label volumes were forced to adapt to a stereotactic space. Each voxel in the probability map for a given anatomical structure describes the probability that the voxel belongs to this anatomical structure. The label volume retrieved from a given threshold is the collection of voxels with probabilities greater than the threshold.

## Labeling the average brain

The anatomical labels were firstly retrieved from the probability map with the threshold that retrieved volumes close to, but less than the population average. These labels were smaller than actual sizes of the corresponding structures, resulting in gaps along the boundaries between neighbor structures. These gaps were filled manually based on the intensity average image. If the boundary could not be easily discriminated on the image, delineations were made along the midpoints between boundaries defined by labels retrieved from the probability map.