ELSEVIER

Journal of Neuroscience Methods 139 (2004) 209–215

JOURNAL OF NEUROSCIENCE METHODS

www.elsevier.com/locate/jneumeth

A standardized method for brain-cutting suitable for both stereology and MRI-brain co-registration

C. Zarow [a,*], T.-S. Kim [b], M. Singh [c], H.C. Chui [d]

[a] *Department of Neurology, Rancho Los Amigos National Rehabilitation Center, University of Southern California, 7601 E Imperial Hwy., Medical Science Building, Room 26, Downey, CA 90242, USA*

[b] *Department of Biomedical Engineering, College of Electronics and Information, Kyung Hee University, 1 Seochun, Kiheung, Yongin, Kyungki 449-701, South Korea*

[c] *Departments of Biomedical Engineering and Radiology, University of Southern California, 3650 S. McClintock Ave, OHE500, Los Angeles, CA 90089-1451, USA*

[d] *Department of Neurology, University of Southern California, Los Angeles, CA 90033, USA*

Received 16 March 2004; received in revised form 29 April 2004; accepted 29 April 2004

Abstract

We have developed an agar-embedding method for brain-slicing that minimizes the geometrical distortions which arise from handling and slicing the fixed postmortem brain. To facilitate postmortem brain-magnetic resonance imaging (MRI) co-registration, each hemisphere is processed separately. We embed the fixed brain hemisphere with reference markers in agar. The block containing the brain and markers is sliced at a fixed interval using a rotary slicer. Each slice is photographed with a high-resolution digital camera. The digital images are realigned as a 3-dimensional volume via a control point-based registration method for multi-slice registration. The realigned multiple slices of the reconstructed postmortem hemisphere are then co-registered to corresponding slices of an in vivo reference MRI-volume. We illustrate these postmortem MRI-brain co-registration methods to correlate in vivo T_2-weighted MRI hyperintensities in gray and white matter with underlying pathology. For design-based stereology, the volume of interest (VOI) is defined using reproducible anatomical boundaries. This method is suitable for stereologic measures of structures ranging from defined nuclei to whole brain.

Keywords: MRI; Brain; Postmortem; Stereology; Co-registration

1. Introduction

Magnetic resonance imaging (MRI) has become increasingly commonplace in the clinical evaluation of dementia. Significant MRI abnormalities include hippocampal atrophy, neocortical atrophy, and focal hyperintensities in subcortical gray and white matter. Kario et al. (1996) in a study of asymptomatic patients with one or more cardiovascular risk factors identified lacunar infarcts on MRI in 53% of those aged 60 or older. Of these, 66% were located in the basal ganglia. Small lacunar infarcts, old, small hemorrhages, and dilated perivascular spaces may all resemble each other radiologically, but the guidelines available to differentiate between lacunar infarcts and dilated perivascular spaces or other lesions on a clinical MRI vary in their recommendations (Kario et al., 1996; Longstreth et al., 1996; Michel and Cruz-Orive, 1988), and none have been specifically validated by MRI-pathological correlation.

Pathological examination provides the gold standard for differentiation of a lacunar infarct (LI) from a dilated perivascular space (PVS). Typically, a LI is an irregular cavity containing strands of fibrillar connective tissue, sometimes enclosing a tiny artery or vein. A dilated PVS, on the other hand, reveals a patent blood vessel, a clearly demarcated wall, and normal surrounding brain tissue (Pullicino et al., 1995). However, a subset of cystic lacunes (presumably large) is filled with fluid and, like PVS, may be isointense relative to CSF. PVS, although usually <2 mm in diameter, may exceed 5 mm particularly near the anterior commissure or the infra-putaminal region, where multiple lenticulostriate arteries course together and turn sharply dorsally (Pullicino et al., 1995). Thus, neither signal characteristics nor size can be completely relied upon for differentiation.

* Corresponding author. Tel.: +1-562-401-7100; fax: +1-562-401-7100.
E-mail address: zarow@usc.edu (C. Zarow).

0165-0270/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2004.04.034

Several challenges are encountered in correlating MRI with postmortem brain. The interval between last clinical MRI and death, postmortem deformation of the brain, and differences in section thickness and orientation hinder correlations. In order to circumvent the delay between MRI and death, some investigators have proposed postmortem MRI (Grafton et al., 1991; Munoz et al., 1993) and some have designed specialized brain slicers (Bronge et al., 2002; Grate et al., 2003; Pullicino et al., 1995). Unfortunately, MRI signal characteristics can change postmortem, making meaningful clinical comparisons difficult. In addition, at the time of death, the brain is subject to multiple deformations. The brain may swell during the agonal state and shrink during fixation. Upon removal from the cranial vault, the ventricles collapse and the brain deforms. The plane-of-section and the thickness of slices usually differ between the brain and MRI, and between consecutive brain slices. The free brain slices lose their orientation with respect to each other and portions of the brain such as the temporal pole fall free of the slice. Identifying the pathological substrate underlying lesions visualized by in vivo MRI is limited by these distortions. We introduce a method for computerized co-registration as a novel tool for imaging-pathological correlations.

Co-registration of the in vivo MRI to postmortem brain for MRI-pathological correlations, as well as sampling of brain tissue for stereological measures, require precisely spaced slices of the human brain. We have developed an agar-embedding method that minimizes the physical distortions usually associated with slicing and handling of the postmortem brain. This method can also be used to embed and slice previously cut material. Incorporation of external fiducials in the agar surrounding each brain slice enables three dimensional reconstruction of the postmortem brain. The reconstructed brain can then be co-registered to the clinical MRI using the specially developed co-registration method which uses polynomial warping and slice-to-volume transformation (Kim et al., 2000, 2001, 2002). In order to reduce the number of distortions within and between slices, and to facilitate MRI co-registration, each cerebral hemisphere is processed separately. An abbreviated form of this method has been previously published (Zarow et al., 2001).

2. Materials and methods

2.1. MRI acquisition protocol

MRIs were performed on a 1.5 T GE Signa system with the following sequences: a T_1-weighted coronal SPGR study angulated orthogonal to the optic nerve with 1.4 mm thick partitions (i.e. slices) and a 1 mm × 1 mm in-plane resolution, TR/TE = 10/4, flip angle = 15°, and a T_2-weighted spin–echo study angulated identically to the SPGR study, flow compensated, 3 mm thick slices, a 50 mm saturation band caudal to MRI slices to suppress inflowing blood; TR/TE = 3400/20/80, acquired as two interleaved data sets to avoid cross-talk between adjacent slices. Serial MRIs were acquired 2–4 years apart. The most recent MRI was used for co-registration with the postmortem brain.

2.2. Tissue preparation

At the time of death, the brain was removed, weighed, photographed, and examined for grossly visible pathology. The entire brain including the brainstem and circle of Willis was suspended in 10% neutral buffered formalin and fixed for a minimum of 10 days. After fixation, the brainstem was removed above the third cranial nerve and the hemispheres were separated at the corpus callosum.

2.3. Mold preparation

The mold assembly is comprised of the mold, the fiducial frame, and fiducial rods (Fig. 1). Plastic food-storage containers (Rubbermaid®-type) make suitable molds and can be found at grocery, warehouse, or dollar stores. The mold must be large enough to accommodate the hemisphere (or whole brain if desired) and the fiducial frame. Currently, we use a mold which is 21 cm × 16 cm × 15.5 cm (L × W × H). A mold with straight sides is ideal. The fiducial rods must be parallel to each other and so are held in place with a frame. We find that plastic 96-well tissue culture plates provide an excellent frame and offer the flexibility of adjusting the placement of the fiducial rods as needed. A minimum of six fiducial rods are used to provide an adequate number of fiducials for later image alignment and registration. Fiducial rods must be rigid, but can be made of wood, plastic or glass. Wooden dowels, plastic rods or glass pipettes are all suitable.

2.4. Mold assembly

The fiducial rods, which will create fiducials in the solidified agar, are placed below and above the tissue, oriented parallel to the sagittal plane. Three fiducial rods are fitted into each side of the frame and the frame is positioned within the mold. One hemisphere is placed in the mold so that it rests on these rods. If desired, the hemisphere can be oriented so that the anterior commissure–posterior commissure (AC–PC) line is parallel to the rods. The three upper fiducial rods are then put in place above the tissue. The mold is labeled to distinguish front (anterior) from back (posterior).

2.5. Tissue-embedding and slicing

The brain is embedded in a solution of 3% agar (Fisher Scientific BP1423) prepared by heating with stirring until the agar is dissolved. The hot agar is tinted with commercially available food coloring (McCormick & Co, Inc. Hunt Valley, MD) to improve the contrast between the brain and the agar. 12 drops of blue and one drop of red are added per 1000 ml of solution. The tinted agar solution is cooled to 85 °C and

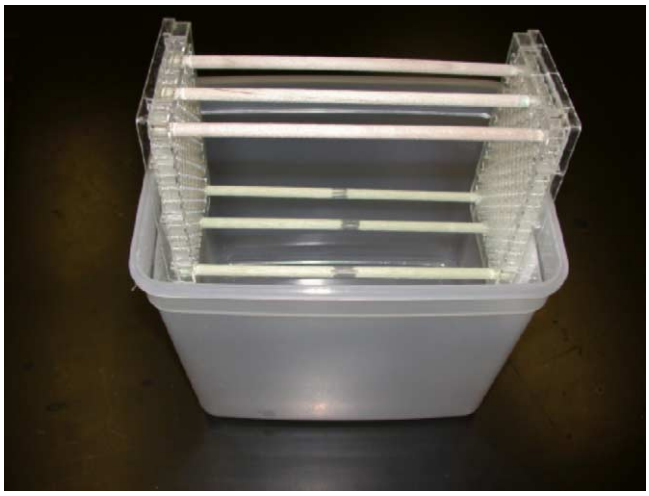

Fig. 1. Mold arrangement illustrating fiducial rods fitted into the holes of 96-well tissue culture plates. With the brain hemisphere positioned between the two sets of rods, the assembly is lowered into the mold.

poured into the mold to completely cover the specimen. The agar is allowed to cool at room temperature or at 4 °C for several hours or overnight. After the agar has solidified it will be opaque. A notch is cut in the top of the agar to mark the orientation. The agar block is removed by inverting the mold. The fiducial frame and fiducial rods are removed, leaving readily visible holes to serve as fiducials. Excess agar is trimmed away by hand.

The firm agar block containing the brain is sliced at fixed intervals using a commercially available, restaurant-style, rotary slicer. The slices are arranged on black Styrofoam trays (Merchants Paper Co., Portland, OR) and photographed with labels, rulers, and color bars in-frame using a high-resolution digital camera (Kodak DC260, 1536 by 1024 resolution) and stored in digital format for MRI co-registration (Fig. 2). The agar surrounding each slice is removed by hand.

2.6. Preparation of hand-sliced material

Archival brains can also be used for design-based stereology. An entire archival hemisphere, previously cut into 1–2 cm thick slices, can be re-stacked, embedded in agar, and sliced on the rotary slicer. Hand-cut slices are stacked and matched against each other. If desired, excess tissue can be trimmed away to allow the agar to surround the region of interest. The stacked block is embedded in tinted agar, with or without fiducials, and sliced as described.

2.7. Postmortem brain image realignment

The series of digital postmortem coronal brain images are realigned to each other by matching the fiducials with a control point-based registration algorithm (Singh et al., 1979). The background including agar and fiducials is removed with pixel intensity thresholding, leaving only the

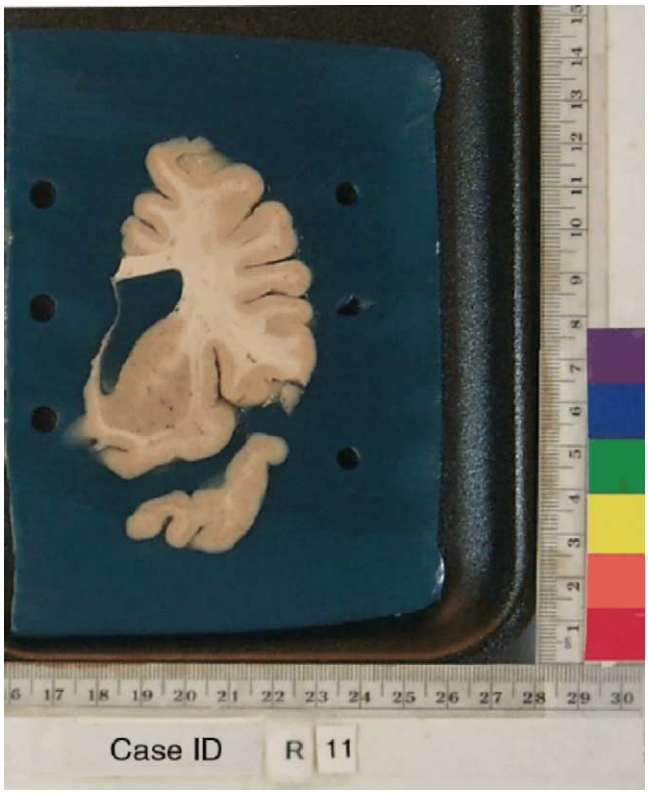

Fig. 2. Embedded brain sliced at 5 mm. Note the relationship of the temporal lobe to the cerebrum. Holes in agar are the fiducials created by the rods. Each image includes case identification, hemisphere designation, slice number, orthogonal rulers, and a color bar in-frame.

brain in the image. To aid the background segmentation in certain cases, image-editing tools such as those found in Adobe Photoshop® are used. Postmortem RGB images are then converted to grayscale images by eliminating hue and saturation while retaining the luminance. To match the orientation of MRI which follows the radiology convention, the background-removed postmortem images are swapped left-to-right.

Finally, a stack of realigned postmortem brain slices is reconstructed as shown in Fig. 3.

2.8. Postmortem MRI-brain co-registration

An in vivo MRI (3D T_1-weighted coronal SPGR, 1.4 mm slice thickness) serves as the reference volume both for T_2 and proton-density coronal MRI and for the postmortem brain (Kim et al., 2000, 2001). The slices of the reconstructed postmortem hemisphere are co-registered to the in vivo reference MRI volume by deriving slices from the MRI-brain volume that match given postmortem brain slice images. This co-registration method uses general *n*-th order polynomial warping to compensate geometrical distortions in postmortem brain slices and the slice-to-volume coordinate transformation such that multiple slice-to-slice matching is achieved rather than volume-to-volume matching. As a registration cost, two intensity similarity measures

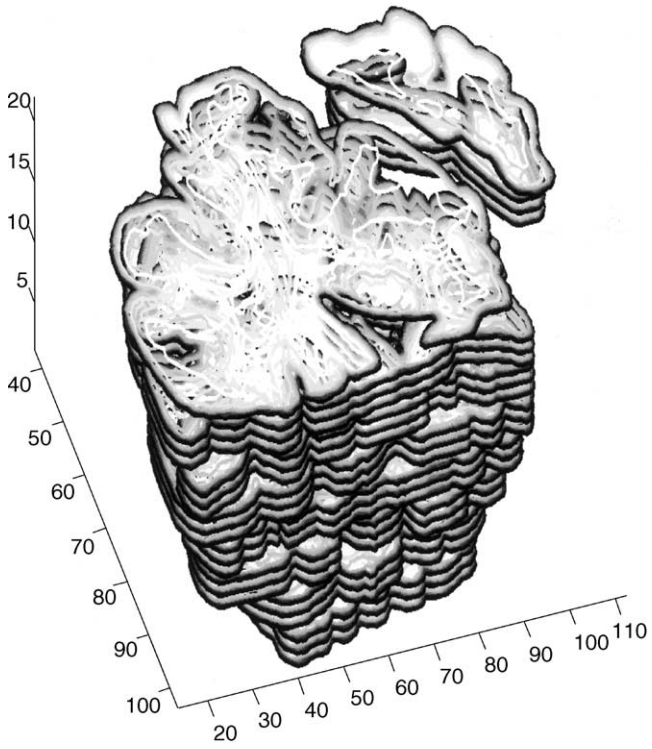

Fig. 3. Image of postmortem slices after converting images to grey scale and digitally removing background and performing fiducial-matching realignment. The gap between the slices is due to the finite thickness of each slice.

are used sequentially: Pearson Cross Correlation (PCC) and Mutual Information (MI) (Kim et al., 2000, 2001, 2002). Once co-registration is achieved, lesions found in MRI or postmortem images are mapped into opposite images (Kim et al., 2003).

3. Results

3.1. Stereology

This brain-cutting technique produces a series of evenly spaced coronal slices of an entire hemisphere or an entire brain suitable for systematic sampling as required for design-based stereology. Tissue blocks taken from the slices can be sectioned on a sledge microtome for thick sections (>50 μm) or embedded into paraffin for sub-serial sampling of the ROI. The thickness of the agar-embedded slices can be tailored to the ROI. For stereological assessments of the numbers of neurons in the hippocampus and cortex and the number of oligodendrocytes, astrocytes, and microglia in the white matter, we use 5 mm thick slices.

3.2. Postmortem MRI-brain co-registration

The co-registration approach relies upon a two-step procedure where initial co-registration is achieved using the PCC measure by finding the images where the overall size and shape of the brain match, followed by a MI cost-function to match further the internal structures and intensity distribution. This approach was validated by a combination of computer simulation studies and an objective evaluation of the voxel mismatch count (VMC) (Kim et al., 2001, 2002). The VMC (Freeborough et al., 1996) counts pixels whose intensity in the difference images generated by subtracting the co-registered MR images from the corresponding postmortem images is greater than 20% of the mean intensity. An example of four co-registered slices and the corresponding difference images is presented in Fig. 4. The relatively uniform gray shade in the difference images denotes a near-zero intensity difference and positive and negative differences appear as bright and dark regions, respectively.

The difference images provide an objective tool to document improvements in the imaging and co-registration methodology and will be used to assess the proposed local nonlinear approaches in the future. Other objective approaches such as those derived from multiple blinded observers to correlate imaging to pathology were considered beyond the scope of the current work.

A representative set of co-registered postmortem and MR images is shown in Fig. 5. To co-register multiple postmortem brain slices simultaneously to their matching MR images, a stack of postmortem images was prepared as discussed above. All slices within the stack were registered with the augmented cost measure PCC followed by MI. The co-registered MRI exhibits close matches in the outer and inner contours of the brain, and the size and shape of the internal structures of the brain including the ventricles, subcortical grey nuclei, corpus callosum, and hippocampus.

3.3. Identification of MRI lesions in the postmortem brain

Once co-registration is achieved, co-registered images are used to correlate hyperintensities visualized by T_2-weighted or proton density MRI with underlying histopathology. White and gray matter hyperintensities are presumed by clinicians to represent ischemic lesions. Lacunes and dilated perivascular spaces can be difficult to distinguish from each other on a clinical MRI. Lesions identified on the MRI by the neuroradiologist are digitally marked by the neuroradiologist on the MRI. The marked MRI is subsequently co-registered to the postmortem brain. The region of brain is then sectioned and the stained slides are reviewed by the neuropathologist (Fig. 6).

4. Discussion

We have developed a method for cutting the human brain into evenly spaced slices suitable for MRI co-registration or stereological measures. The method is simple and inexpensive to set up and yields high quality images suitable for the studies described herein. In a series of 11 sequential autopsies for which pre-mortem MRI was available, one was

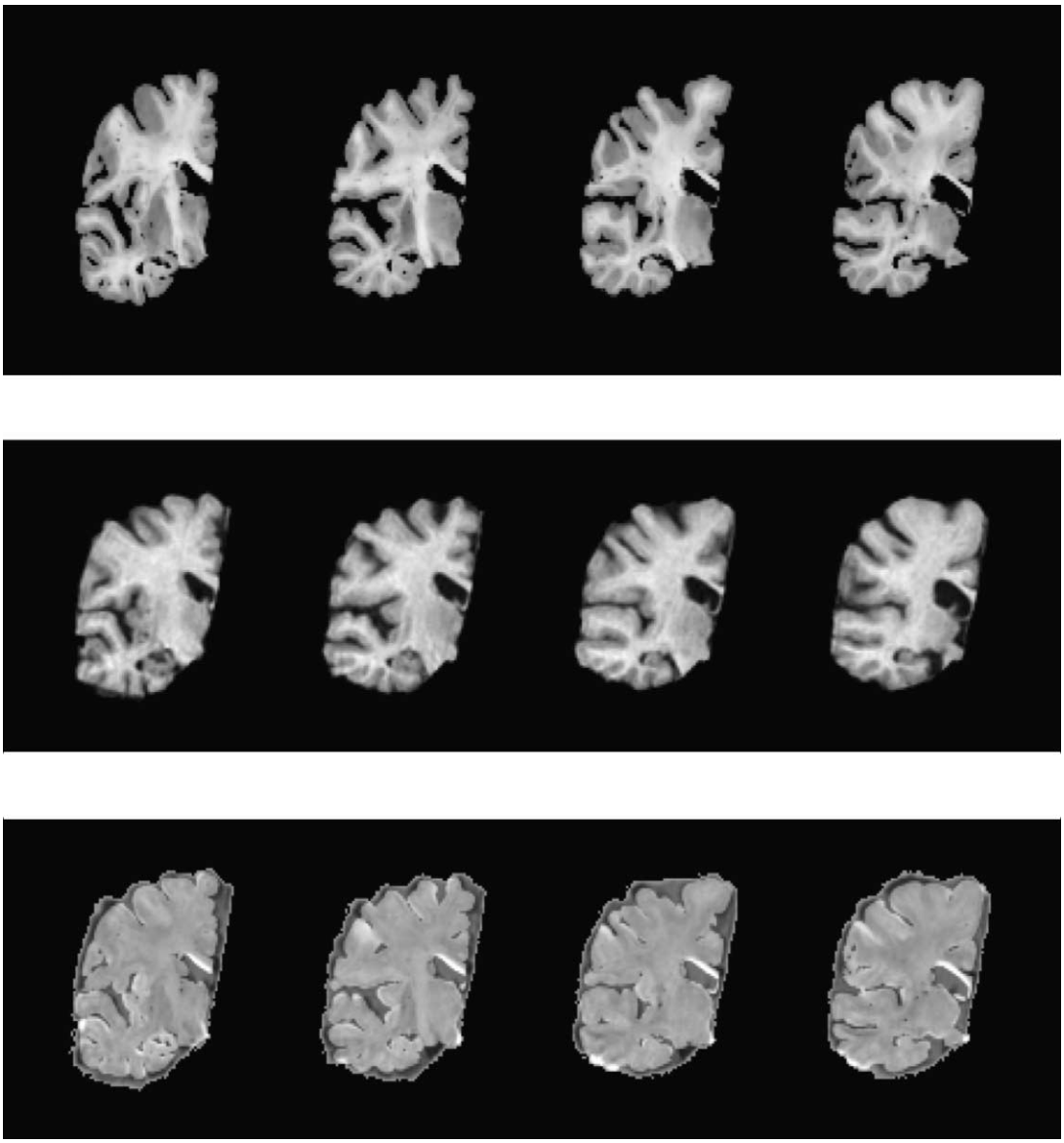

Fig. 4. Top: four consecutive postmortem brain slices from a 92-year-old female. Middle: slices from MRI performed 1.5 years prior to death registered using PCC + MI. Bottom: top images subtracted from middle images to give difference images (top) minus (middle).

found not to be suitable for co-registration due to movement in the MRI. The remaining 10 cases, which included diagnoses of Alzheimer disease, cerebrovascular disease, and pathologically normal cases, were co-registered successfully. The time between MRI and death ranged from 0.31 to 5.38 years (average 2.06 ± 1.96 years). Average age of cases was 89.7 ± 5.2 years. Brain weight averaged 1092 ± 72 g. Neither brain atrophy nor significant numbers or volumes of vascular lesions compromises this method. Indeed, the handling of pathological material is made easier by embedding it in a matrix. The presence of fiducials eliminates the need to precisely orient the tissue to any outside metric.

We reasoned that while the postmortem brain could only be cut once, the MRI could be digitally re-sliced an infinite number of ways. Therefore, we chose the reconstructed postmortem brain images as the template to be matched, and re-sliced and warped the MRI to match the reference postmortem slices. The best results were obtained when multiple warped slices were withdrawn from the MRI volume to match several serial postmortem brain slice images simultaneously.

Other investigators have attempted to match the postmortem brain to premortem or postmortem MRI. Grate et al., in a study of traumatic brain injury in piglets, used a specially-designed cutting frame to approximate the orientation of the cut brain to the MRI. MRI images were then simply compared visually to cut and stained histological sections (Grate et al., 2003). In a study of white matter changes in Alzheimer disease, a specially-designed cutting frame was also used by Bronge et al. (2002) to approximate

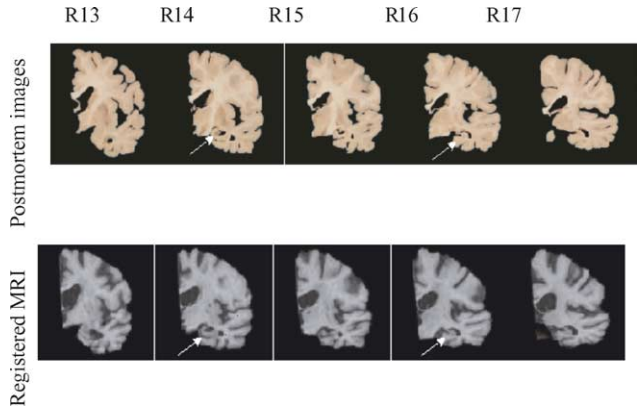

Fig. 5. Simultaneous co-registration of premortem MRI to five serial slices of the postmortem brain in an 83-year-old male with Alzheimer disease. Top row indicates slice number of the right hemisphere. Close matches are seen in both the outer contour of the images and the subcortical structures. Note especially the close co-registration of the hippocampus (arrows).

the orientation of the cut brain to the postmortem MRI. Jacobs et al., used surface matching registration and non-linear thin plate spline warping techniques to warp MRI to histological sections in a study of experimentally induced focal cerebral ischemia in rat (Jacobs et al., 1999). Mega et al. (1997) undertook to register whole-brain stained sections and premortem functional imaging with a digitally reconstructed postmortem brain volume. A 3D reference volume was created from digital images of slices of the postmortem brain taken before sectioning. Premortem imaging (FDG–PET) was co-registered to the reference volume using a six-parameter rigid body algorithm. Stained sections were warped back to the reference volume using a 3D elastic warping algorithm. Unfortunately, histological assessment was limited by freezing artefact.

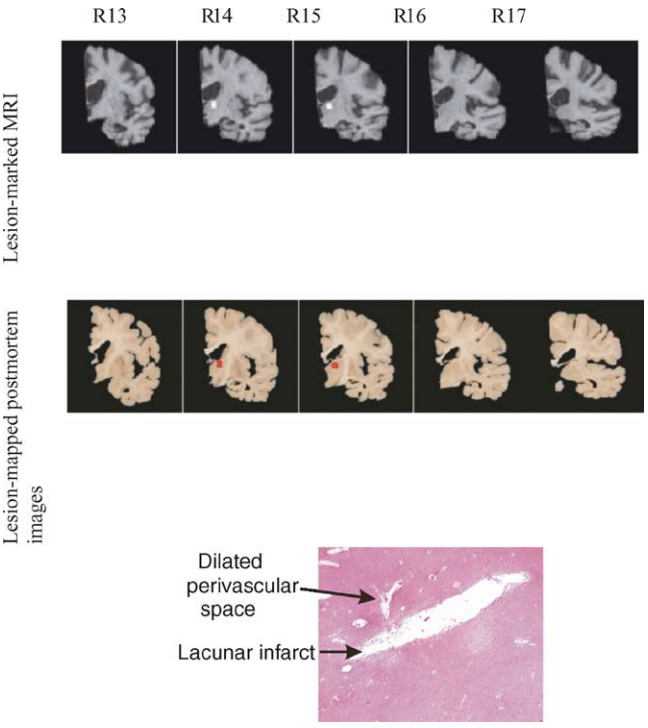

Fig. 6. Registration results of multiple postmortem brain slices to their matching MRI slices in the same case as Fig. 5. Top: same series of MRI images as in Fig. 5. Abnormalities identified by the neuroradiologist are digitally marked on each MRI image, seen here as white marks. Middle: marked MRIs are mapped to the postmortem brain slices (red). Bottom: H and E stained section containing the region marked in R15. The neuropathologist identified a slit-like lacunar infarct and a dilated perivascular space in this region.

This method offers several advantages over previous methods. Agar provides a semi-rigid matrix suitable for embedding the postmortem brain. It can be tinted to enhance the contrast between tissue and matrix. This method produces regularly spaced slices which cannot be had by hand-cutting methods. The brain can be sliced in the coronal, sagittal, axial, or randomly oriented plane. Portions of the brain which might be lost or displaced during hand-cutting retain their proper anatomical relationships. It can be adapted for small animal brains simply by scaling down the size of the mold and fiducial frame. Systematic serial slices of the entire brain or sub-structure are suitable for design-based stereological studies. Fiducial markers facilitate 3D reconstruction of the postmortem brain images which can then be co-registered to the clinical MRI. However, the further identification of lesions seen in the MRI, and subsequently sought out in the postmortem brain, is limited by the resolution of the MRI images, currently only a few millimeters. Further improvements in imaging and warping techniques, such as the incorporation of local nonlinear corrections, will serve to enhance our ability to match small lesions more accurately. Identification of the pathological structures by a neuropathologist provides a clear validation of this technique.

Acknowledgements

Supported by NIH P01-AG12435A.

References

Bronge L, Bogdanovic N, Wahlund LO. Postmortem MRI and histopathology of white matter changes in Alzheimer brains. A quantitative, comparative study. Demen Geriatr Cogn Disord 2002;13:205–12.

Freeborough PA, Woods RP, Fox NC. Accurate registration of serial 3D MR brain images and its application to visualizing change in neurodegenerative disorders. J Comp Assist Tomogr 1996;20:1012–22.

Grafton ST, Sumi SM, Stimac GK, Alvord EC, Shaw CM, Nochlin D. Comparison of postmortem magnetic resonance imaging and neuropathologic findings in the cerebral white matter. Arch Neurol 1991;48:293–8.

Grate LL, Golden JA, Hoopes PJ, Hunter JV, Duhaime AC. Traumatic brain injury in piglets of different ages: techniques for lesion analysis using histology and magnetic resonance imaging. J Neurosci Methods 2003;123:201–6.

Jacobs MA, Windham JP, Soltanian-Zadeh H, Peck DJ, Knight RA. Registration and warping of magnetic resonance images to histological sections. Med Phys 1999;26:1568–78.

Kario K, Matsuo T, Kobayashi H, Asada R, Matsuo M. 'Silent' cerebral infarction is associated with hypercoagulability, endothelial cell damage, and high Lp(a) levels in elderly Japanese. Arterioscler Thromb Vasc Biol 1996;16:734–41.

Kim TS, Singh M, Ghugre N, Kim S, Zarow C, Chui HC. Registration of postmortem brain slices to matching MR-slices within 3-D reference MRI. Int Soc Magn Reson Med 2001;9:814.

Kim TS, Singh M, Ghugre N, Kim S, Zarow C, Chui HC. MRI-guided histological analysis of postmortem brain slices. Int Soc Magn Reson Med 2003;11:2255.

Kim TS, Singh M, Kim S, Zarow C, Chui HC. Simultaneous registration of multiple postmortem brain slice images to their corresponding MRIs. Int Soc Magn Reson Med 2002;10:2486.

Kim TS, Singh M, Sungkarat W, Zarow C, Chui HC. Automatic registration of postmortem brain slices to MRI reference volume. IEEE Trans Nucl Sci 2000;47:1607–13.

Longstreth WT, Manolio TA, Arnold A, Burke GL, Bryan N, Jungreis CA, Enright PL, O'Leary D, Fried L. Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study. Stroke 1996;27:1274–82.

Mega MS, Chen SS, Thompson PM, Woods RP, Karaca TJ, Tiwari A, Vinters HV, Small GW, Toga AW. Mapping histology to metabolism: co-registration of stained whole-brain sections to premortem PET in Alzheimer's disease. Neuroimage 1997;5:147–53.

Michel RP, Cruz-Orive LM. Application of the Cavalieri principle and vertical sections method to lung: estimation of volume and pleural surface area. J Microsci 1988;150:117–36.

Munoz DG, Hastak SM, Harper B, Lee D, Hachinski VC. Pathologic correlates of increased signals of the centrum ovale on magnetic resonance imaging. Arch Neurol 1993;50:492–7.

Pullicino PM, Miller LL, Alexandrov AV, Ostrow PT. Infraputaminal 'lacunes'. Clinical and pathological correlations. Stroke 1995;26:1598–602.

Singh M, Frei W, Shibata T. A digital technique for accurate change detection in nuclear medicine images with applications to myocardial perfusion studies using thallium-201. IEEE Trans Nucl Sci 1979;26:565–75.

Zarow C, Kim TS, Chui HC. A standardized method for brain-cutting suitable for both MRI-brain co-registration and stereological measures. Society For Neuroscience Abstracts, 27, Program number 426.2, 2001.