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CT Guidance is Needed to Achieve Reproducible Positioning of the Mouse Head for Repeat Precision Cranial Irradiation

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Abstract

To study the effects of cranial irradiation, we have constructed an all-plastic mouse bed equipped with an immobilizing head holder. The bed integrates with our in-house Small Animal Radiation Research Platform (SARRP) for precision focal irradiation experiments and cone-beam CT. We assessed the reproducibility of our head holder to determine the need for CT based targeting in cranial irradiation studies. To measure the holder's reproducibility, a C57BL/6 mouse was positioned and CT scanned nine times. Image sets were loaded into the Pinnacle³ radiation treatment planning system and were registered to one another by one investigator using rigid body alignment of the cranial regions. Rotational and translational offsets were measured. The average vector shift between scans was 0.80 ± 0.49 mm. Such a shift is too large to selectively treat subregions of the mouse brain. In response, we use onboard imaging to guide cranial irradiation applications that require sub-millimeter precision.

INTRODUCTION

The deployment of advanced technologies and the translation of laboratory discoveries into human treatment must be supported by rigorous preclinical studies. Preclinical studies of new radiation treatment methods, however, have been difficult due to the technological disparity between the simple methods used for laboratory animal irradiation and those for advanced human treatment. Recognition of this deficiency has led us (1) and other investigators (2–5) to develop specialized precise radiation delivery systems to deliver radiation to a localized region in laboratory mice and rats. The scale of the problem can be appreciated when one considers that the brain of the mouse is approximately 1 cm wide and that the structures of interest within the brain are often less than 1 mm in at least one dimension.

Several of the new small animal radiation systems are equipped with on-board CT guidance to facilitate precise positioning in repeat irradiation experiments. Such capability parallels the online image guidance technologies in human treatment, including cone-beam CT (CBCT), which are supplanting the use of custom immobilization devices to set up each patient. On-board imaging is advantageous for human applications to monitor the inherent motion of the unsedated patient and in the case of CBCT to provide information about soft tissue targets (6, 7). However, it remains unclear whether on-board CT imaging is required

for accurate repositioning where the fixation of an anesthetized animal might suffice. The question should be addressed given the significant leap in technology and cost associated with image guidance for small animal irradiation.

MATERIALS AND METHODS

All experiments were performed with the approval of the Johns Hopkins Animal Care and Use Committee. In this study, we focused on the mouse head as the site of interest because its relatively rigid structure and well-defined bony anatomy represent a best-case scenario for setup with a fixation system. A custom-made mouse bed equipped with an immobilizing head holder was designed and constructed to support setup for focal brain irradiation as shown in Fig. 1. The design is based on the mouse stereotactic systems in common use (e.g. Kopf Inc., Tujunga, CA). The main difference is that our holder does not employ ear bars, which were determined to be too difficult and inefficient to use with our mouse bed for the sequential irradiation of many mice. It has a removable bite block for the front teeth and a neck collar made from laser-cut Styrofoam. The arrangement allows for quick setup and rigid immobilization. Isoflurane gas can be delivered via a tube that is integrated into the bite block and emerges in front of the mouse's nose, thus keeping the mouse anesthetized for the duration of a procedure. The all-plastic construction of the device is CT- and MR-compatible and fits into the 30-mm-diameter mouse coil commonly used on the 9.4 T MRI scanner (Bruker Inc., Billerica, MA).

Assessment of the reproducibility of the head fixation system for precision irradiation was conducted with our in-house small animal radiation research platform (SARRP). The SARRP employs a dual-focal spot, constant-voltage X-ray source (Seifert, Fairview Village, PA) for both imaging and irradiation. It is mounted on a gantry with a source-to-isocenter distance of 35 cm. Manual gantry rotation is limited to 120° from vertical, in 15° increments. Robotic translate/rotate stages are used to control the positioning of the animal. Depending on the tissue of interest, X rays of 50 to 100 kVp from the smaller 0.4-mm focal spot are used for imaging. On-board CBCT imaging is achieved by a 2π rotation of the horizontal animal between the stationary X-ray source and a 20 cm × 20-cm flat-panel detector (Perkin Elmer, Santa Clara, CA). The flat panel has 512 × 512 pixels and is positioned to achieve an image magnification of 1.5, resulting in a pixel dimension of 0.26 mm × 0.26 mm at isocenter. For practical reasons, CBCT images are reconstructed at 0.52 mm × 0.52 mm × 0.52 mm voxel resolution. Figure 2 shows a CAD drawing of the CBCT scanning orientation and the resultant 100 kVp images of a mouse head. The CT imaging dose is less than 1 cGy using 100 kVp X rays. We reported previously study that our on-board CBCT facilitated accurate image-guided irradiation of a rigid phantom (i.e. a radio-opaque marker) to within 0.2 mm (8).

A session of setup and CBCT imaging was repeated nine times on one 4-week-old C57BL/6 mouse. The study avoided the variability that could be introduced by the anatomical differences between different mice and focused on the head-holding device itself. Isoflurane gas anesthesia was administered continuously during imaging. For each imaging session, the mouse was immobilized with the head holder, i.e., bite block and head collar, on the supporting bed and setup in the irradiation position on the SARRP. A CBCT was acquired from 360 projection images at an angular spacing of 1° per image. After each scan, the mouse and the bite block were removed from the bed and separated from each other and then the mouse was carefully reimmobilized for the next scan. Because the bed remained unperturbed on the rigid stage, variation in the setup would be largely associated with the positioning of the mouse and the head holder.

The variability of the head position of the mouse, as measured in the nine-CBCT data set, was analyzed using the image fusion utility of the Pinnacle³ Radiation Therapy Planning software v. 8.1y (Philips Inc., Madison, WI). Each CBCT data set contains full 3D volumetric data for the cranial setup. One arbitrary scan was chosen from the nine scans to serve as a reference. Each of the remaining nine scans was aligned to this reference based on 3D rigid body translations and rotations. The alignment between two sets of CBCT images was performed manually on Pinnacle³ at a resolution for translation of 0.1 mm and rotational resolution of 1° increments. Automatic image registration methods were unavailable at the time of our study. During the alignment process, scans were evaluated visually to achieve the best congruence of the landmark bony features of the cranium. The subjective evaluation of the alignment was aided by a variety of checker-box displays in Pinnacle's image fusion software as shown in Fig. 3. One user performed all manual registrations. The same user also performed three repeat manual registrations of each setup of the mouse on three separate days to provide a measure of user-introduced variability. The repeat registrations were performed on three separate days. As a test for accuracy of the registration, the reference scan was loaded and aligned to itself, and the variations of the measured offsets of all alignments were analyzed.

RESULTS

By aligning the bony anatomy of the mouse in each scan with that of the reference scan, we were able to calculate the displacement of the mouse's head position between any two scans. Results were read directly from the Pinnacle³ software. For each alignment of two CT scans, we obtained offset values for translations in the *X*, *Y* and *Z* directions in centimeters and also rotations about each axis in degrees. A vector displacement from the reference image was then calculated as defined by the square root of the sum of squares of the translation in each direction, i.e. ($\Delta X^2 + \Delta Y^2 + \Delta Z^2$). Table 1 shows the average alignment offset data for the mouse study obtained through an average of multiple manual registrations using the Pinnacle³ software. The average displacement of all setups is 0.80 mm with a standard deviation (SD) of ± 0.49 . The average of all measured rotational offsets is 0° with an SD of $\pm 2^\circ$. The large variation of angular offset reflects the coarse one degree resolution used for alignment.

The intrauser variation of the three repeat alignments was significantly smaller than the variation measured for the repeat setups ($P < 0.008$). For all repeat alignments, the average difference is -0.18 ± 0.05 mm in vector translation and $-0.4 \pm 0.9^\circ$ in rotation. For comparison, the self-alignment of the reference CBCT yielded an average offset of 0.1 ± 0.1 mm in the translation vector and $0.0 \pm 0.4^\circ$ in rotation.

DISCUSSION

Our group has constructed a novel focal irradiation system for small animals with on-board CBCT guidance capability (1). Our study shows that while a head holder can produce reasonable reproducibility for repeat setup of the order of 1 mm, on-board CT guidance is clearly necessary if a setup of sub-millimeter accuracy is required. This capability has been demonstrated with the SARRP in idealized phantom irradiation experiments (8) and also in the present study with the mouse brain.

The purpose of any immobilizing device for radiation treatment is to ensure accuracy. The precision required of the SARRP head holder is dictated by the particulars of experimental design. One example application is the study of radiation effects on two functional regions that have been identified in rodents as niches for neuronal proliferation (9, 10): the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the

dentate gyrus (DG) of the hippocampal formation. While much of the radiation administered to small animals in this area of research has been either whole-brain or whole-body (11, 12), some groups have investigated using partial-brain irradiation (13, 14, 15). However, the precision of these irradiation methods has been limited, particularly in view of the minute dimensions of the dentate gyrus of the adult mouse, which has a coronal cross section of roughly 1.25 mm × 0.5 mm, while the lateral ventricle measures about 1.75 mm × 0.85 mm (16). The SARRP, with its ability to irradiate a target to within 0.2 mm accuracy under CT guidance, presents a better approach.

Recent work by Kiehl (17) examined localized hemispherical irradiation of the mouse brain with treatment planning capabilities. The stated total uncertainty of their head-holder-based system was ±0.30 mm. The lower variability is likely due to their use of ear bar fixation. It also has been reported that it is possible to achieve accurate irradiation of rat's brain to within 1 mm by head fixation using a minimally invasive stereotactic device (18). However, greater accuracy is needed to selectively irradiate the smaller functional subregions in the mouse brain such as the DG or SVZ. Fricke *et al.* created an MRI- and CT-compatible stereotactic device for rats that with a reproducibility of about 0.1 mm for imaging studies (19). The design, however, has not been used for radiation treatment. Our earlier attempts showed that the design was less suitable for mice and led to our adapting one that is less restrictive but more efficient.

In summary, we determined that CT guidance was necessary to ensure high-precision repeat irradiation even when the anesthetized animal was immobilized, albeit in a less restrictive device. As a corollary, on-board image guidance ensures accurate irradiation, alleviating the concern of inadequate animal immobilization.

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FIG. 1.

Custom stereotactic mouse head holder. Bite block unit detaches from bed (left) and mouse teeth are clamped in center hole by rubber band. Assembly is inserted into the bed holder (right) and Styrofoam neck collar provides additional stability.

FIG. 2.
 Panel A: Cone-beam CT acquisition geometry with the small animal radiation research platform (SARRP). The X-ray tube and digital detector are fixed while the animal rotates around a ventral-to-dorsal axis. Panel B: Representative cuts are shown from the coronal, sagittal and transverse planes.

FIG. 3.

Image registration of two consecutive CT scans of a C57/BL6 mouse. Images are shown in the sagittal, coronal and transverse planes (panels A, B and C, respectively). The secondary (orange) has been rotated and translated until aligned with the reference scan (black and white).

TABLE 1

Alignment Offsets

Scan	Translation (mm)				Rotation (degrees) about axis		
	ΔX	ΔY	ΔZ	V	X	Y	Z
1	-0.33 (0.15)	0.10 (0.10)	-0.20 (0.00)	0.4 (0.2)	0.1 (0.1)	-1.60 (0.78)	-0.03 (0.05)
2	0.57 (0.23)	-0.23 (0.06)	1.13 (0.06)	1.29 (0.24)	2.63 (0.59)	2.98 (0.83)	-0.37 (1.13)
3	-0.67 (0.06)	-0.17 (0.06)	1.33 (0.06)	1.50 (0.10)	3.32 (0.16)	-2.38 (0.19)	2.13 (0.33)
4	-1.07 (0.21)	0.53 (0.06)	-0.50 (0.00)	1.29 (0.22)	-3.55 (0.12)	-3.47 (1.14)	2.10 (0.35)
5	-0.10 (0.10)	0.47 (0.15)	0.0 (0.1)	0.48 (0.22)	-1.79 (0.87)	0.90 (0.79)	1.05 (0.20)
6	-0.07 (0.06)	0.17 (0.15)	0.40 (0.00)	0.44 (0.16)	0.34 (0.59)	0.51 (0.57)	0.75 (0.67)
7	-0.13 (0.06)	0.13 (0.12)	-0.20 (0.10)	0.27 (0.16)	-1.84 (0.65)	-0.43 (0.62)	0.0 (0.1)
8	-0.20 (0.10)	0.43 (0.06)	-0.50 (0.00)	0.69 (0.12)	-2.04 (0.80)	-0.25 (1.40)	0.91 (0.20)
Mean	-0.25	0.18	0.18	0.80	-0.36	-0.47	0.81
SD	0.48	0.29	0.71	0.49	2.40	2.03	0.94
Self	-0.1 (0.1)	0.0 (0.0)	0.0 (0.0)	0.1 (0.1)	0.1 (0.1)	-0.28 (0.44)	0.21 (0.46)

Notes. The mean offsets and SD for the three repeat alignments of each mouse are shown. After the offsets in the CBCTs were measured, the data were averaged. Means of all measured offsets and their corresponding SD are shown in the bottom rows. Control data for the self-alignments are also shown.