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Breath-hold Device for Laboratory Rodents Undergoing Imaging Procedures

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The increased use in noninvasive imaging of laboratory rodents has prompted innovative techniques in animal handling. Lung imaging of rodents can be a difficult task because of tissue motion caused by breathing, which affects image quality. The use of a prototype flat-panel computed tomography unit allows the acquisition of images in as little as 2, 4, or 8 s. This short acquisition time has allowed us to improve the image quality of this instrument by performing a breath-hold during image acquisition. We designed an inexpensive and safe method for performing a constant-pressure breath-hold in intubated rodents. Initially a prototypic manual 3-way valve system, consisting of a 3-way valve, an air pressure regulator, and a manometer, was used to manually toggle between the ventilator and the constant-pressure breath-hold equipment. The success of the manual 3-way valve system prompted the design of an electronically actuated valve system. In the electronic system, the manual 3-way valve was replaced with a custom designed 3-way valve operated by an electrical solenoid. The electrical solenoid is triggered by using a hand-held push button or a foot pedal that is several feet away from the gantry of the scanner. This system has provided improved image quality and is safe for the animals, easy to use, and reliable.

Abbreviations: CT, computed tomography

Noninvasive imaging of laboratory rodents permits researchers to visualize internal, functional, and morphologic changes in laboratory rodents without having to euthanize the animals; therefore researchers can conduct longitudinal studies using orthotopic and genetic models.[5,6] The unique requirements of the imaging environment often mean that innovative techniques are needed to immobilize animals and control their normal physiologic processes during imaging procedures. For example, normal breathing typically causes motion artifacts in images of the lungs or abdomen. We previously described the use of respiratory-gated imaging of ventilated rodents during imaging procedures using a micro-computed tomography (μCT) system that required periodic acquisition of imaging data over a prolonged time at specific points in the respiratory cycle.[2,4,12]

However, with the development of a prototype flat-panel CT scanner (Figure 1), it became possible to acquire detailed images of the lungs of a mouse or rat in as little as 2, 4, or 8 s and of those in a dog in approximately 40 s.[9] This single, short, continuous image acquisition precludes the use of respiratory gating and requires a single static breath-hold to inflate the lungs and hold them in an inflated state during the 2- to 8-s image acquisition. We have built a device that safely expands the lungs and holds them at specific pressures by using 100% oxygen administered at a constant pressure during the image acquisition process. Using this device, we are able to successfully perform routine breath-holds on mice and rats during imaging acquisitions. This ability to sustain a breath-hold has minimized breathing motion artifacts and thus improved the quality of the images acquired with this instrument.

Another source of artifact is cardiac motion. Cardiac motion can have a substantial effect on surrounding lung tissue.[1] Although cardiac motion blurring appears to be reduced when using a breath-hold data acquisition approach compared with a free-breathing acquisition, some cardiac motion-induced blur remains in the breath-hold images. With the minimum 2-s rotation time of the flat-panel CT scanner, cardiac gating of small laboratory animals with relatively high physiologic heart rates is not considered feasible at this time. Improvements in technology and further study will be required to incorporate cardiac gating into CT imaging of rodents by use of the flat-panel CT scanner.

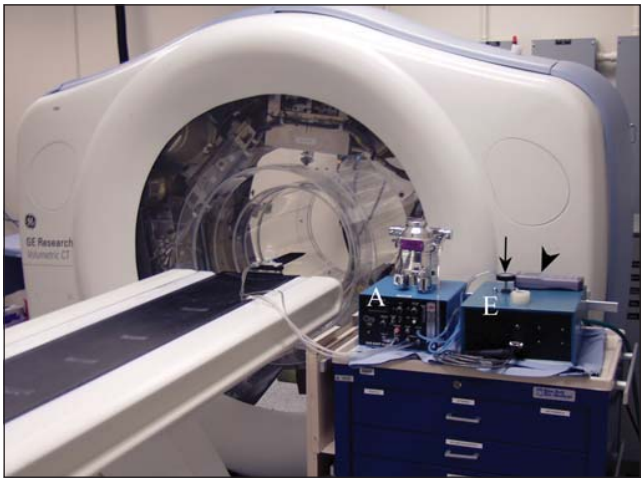

Figure 1. Prototype flat-panel computed tomographic scanner set up with small-animal ventilator (A), electronic breath-hold device (E), adjustable pressure regulator (arrow), and pressure manometer (arrowhead).

The flat-panel CT scanner we used is a preclinical experimental prototype CT scanner that was developed exclusively for animal studies. The scanner represents a compromise between the speed of a clinical CT scanner and the resolution of an animal scanner. The scanner uses dual digital flat-panel X-ray detectors

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coupled to a standard high-performance CT X-ray tube. The X-ray source–detector assembly is attached to a standard CT gantry with slip-ring technology and is equipped with a standard CT patient table.[4] This scanner provides spatial resolution in the range of 170 to 250 µm, which is a several-fold improvement over that of clinical CT scanners, and the Z-axis coverage per rotation is 4.21 cm. The speed of acquisition (2, 4, or 8 s per rotation) distinguishes this scanner from most commercially available µCT scanners. The short acquisition time of this experimental scanner also permits the use of standard iodinated intravenous contrast material for imaging studies requiring the use of contrast, whereas the prolonged scan times of the typical µCT scanner typically precludes the use of this type of contrast reagent and requires special blood pooling agents.

Little published information is available on breath-hold procedures in laboratory rodents undergoing CT imaging, although there are publications that describe the use of breath-holds in mice,[3] rats,[7] rabbits,[10] dogs,[11] and pigs[13] during magnetic resonance imaging. Breath-hold imaging CT studies at our institution typically are performed on mice and rats. We looked at several models of small-animal positive-pressure mechanical ventilators and were unable to find a commercially available ventilator that was designed to hold a constant inspiratory air pressure for a specific amount of time. Commercially available ventilators typically have a 'hold' or 'sigh' action, but this feature only stops the current ventilation protocol and continually adds or releases air to the lungs, resulting in constant tissue motion. In addition, 1 commercially available small-animal system needed to be within 1 foot of the animal; this configuration would not be desirable in most imaging situations. As a result, we developed this novel breath-hold device that works separately from our existing ventilator and allows uniform, consistent expansion of the lungs at a constant pressure.

Materials and Methods

All laboratory rodents were anesthetized and intubated using a method described in a previous manuscript.[8] Briefly, mice were intubated with standard disposable 20-gauge, 1-in. intravenous catheters (Ethicon, Endo-Surgery, Cincinnati, OH). Rats were intubated with 16- or 18-gauge catheters (Angiocath, BD Infusion Therapy Systems, Sandy, UT), depending on their body size. The larger catheters for rats were measured individually from mouth to thoracic inlet and cut to the proper length. Healthy mice and rats were preanesthetized with subcutaneous injection of 1.0 mg/kg medetomidine hydrochloride (1.0 mg/ml; Dormitor, Orion, Espoo, Finland) and 0.4 mg/kg atropine sulfate (0.4 mg/ml; American Pharmaceutical Partners, Los Angeles, CA). After 2 to 3 min, the rodent was placed into an induction chamber, where it was anesthetized with 2% isoflurane and an oxygen flow rate of 2 l/min. Once the rodent was unconscious, it was removed from the induction chamber and placed at a procedure station, where its respiratory rate could be monitored. When the respiratory rate (assessed by visual observation), decreased to approximately 30 breaths/min for mice and 20 breaths/min for rats, the anesthetic state of the rodent was suitable for intubation. The rodent was placed on an intubation stand, and a small-animal intubation illumination system (BioLite, BioTex, Houston, TX) was used for intubation. Once intubated, the rodent was placed supine onto a half-pipe acrylic sled and connected to a small-animal ventilator (SAR-830/AP, Charles Ward Enterprises, Ardmore, PA). For mice, the ventilator was set at a breathing rate of 90 to 110 breaths/min, a pressure of 16 cm H_20, and an oxygen flow rate of 250 ml/min. For rats, the ventilator was set at a breathing rate of 50 to 60 breaths/min, a pressure of 16 to 18 cm H_20, and an oxygen flow rate of 550 ml/min. Anesthesia was maintained with 1.0% to 2% isoflurane throughout the imaging session. Once imaging was complete, the rodent received a subcutaneous injection of 2.5 mg/kg atipamezole hydrochloride (5.0 mg/ml; Antisedan, Orion) to reverse the medetomidine dose. When the mouse or rat started to breathe on its own, it was extubated.

For the purpose of this study, the imaging session for mice required approximately 3 min which included a scout image for positioning and a breath-hold image. If a contrast-enhanced image was required, 2 min was added to the total imaging time to acquire the post-contrast image data. For rats, total imaging time was the same as for mice. Image acquisition parameters for mice were: rotation time, 8 s; exposure time, 8 s; 100 kV; 30 mA; and 1000 views/rotation with coverage of 13 (X axis) × 13 (Y axis) × 4.21 (Z axis). For rats the imaging parameters were: rotation time, 8 s; exposure time, 8 s; 100 kV; 60 mA; and 500 views/rotation with coverage of 13 (X axis) × 13 (Y axis) × 4.21 (Z axis). Data were reconstructed with the standard reconstruction filter within the reconstruction software, and data was visualized with MicroView software (version 2.0, GE Healthcare, London, Ontario, Canada). For the contrast study, 200 µl of a 1:4 dilution (with 0.9% sodium chloride) of a standard iodinated agent (678 mg ioversol/ml, Optiray, Mallinckrodt, Hazlewood, MO) was injected intravenously over 3 s. We incorporated a 10-s delay, which began after the injection and ended just before the start of image acquisition, into the imaging protocol.

The protocols for treatment of all animals used in the development and testing of this breath-hold device were reviewed and approved by our institutional animal care and use committee. This procedure was developed using outbred ICR mice (Harlan Sprague-Dawley, Indianapolis, IN) and inbred Fisher rats (Charles River Laboratories, Wilmington, MA). All animals were housed conventionally and maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International, in the Department of Veterinary Medicine and Surgery at The University of Texas MD Anderson Cancer Center (Houston, TX).

Our initial breath-hold device comprised a manually operated 3-way valve, an air pressure regulator, and a manometer. A standard, commercially available Luer lock 3-way valve (Qosina, Edgewood, NY) initially was used for manual toggling between the ventilator and the breath-hold system. The animal, ventilator, and constant-pressure breath-hold device were connected to the Luer lock connections of the 3-way valve. Because of the small pulmonary tidal volume in mice, we modified the Luer lock connections between the mouse and the ventilator and the 3-way valve by machining down the male portions of the Luer lock connections to allow a closer fit and reduce the amount of dead space resulting from the connections.

An air-pressure regulator (Control Air, Amherst, NH) was placed between the oxygen source and the 3-way valve to allow the pressure of the oxygen source for the breath-hold to be reduced to an appropriate level (Figure 2). Typically, this regulator is set to reduce the pressure of the 100% oxygen gas that is used during the breath-hold of rodents to a level of 16 cm H_2O. In a previous study, this pressure resulted in the best tissue contrast without distention of bronchi.[2] However, because of the flexibility of this system, the regulator can be set to different pressures, allowing investigators a wide range of lung pressures for their research studies. A manometer (Control Company, Friendswood, TX) was connected to the air-pressure regulator, allowing the pressure of the oxygen to be set and monitored during the breath-hold. Before the system was used on an

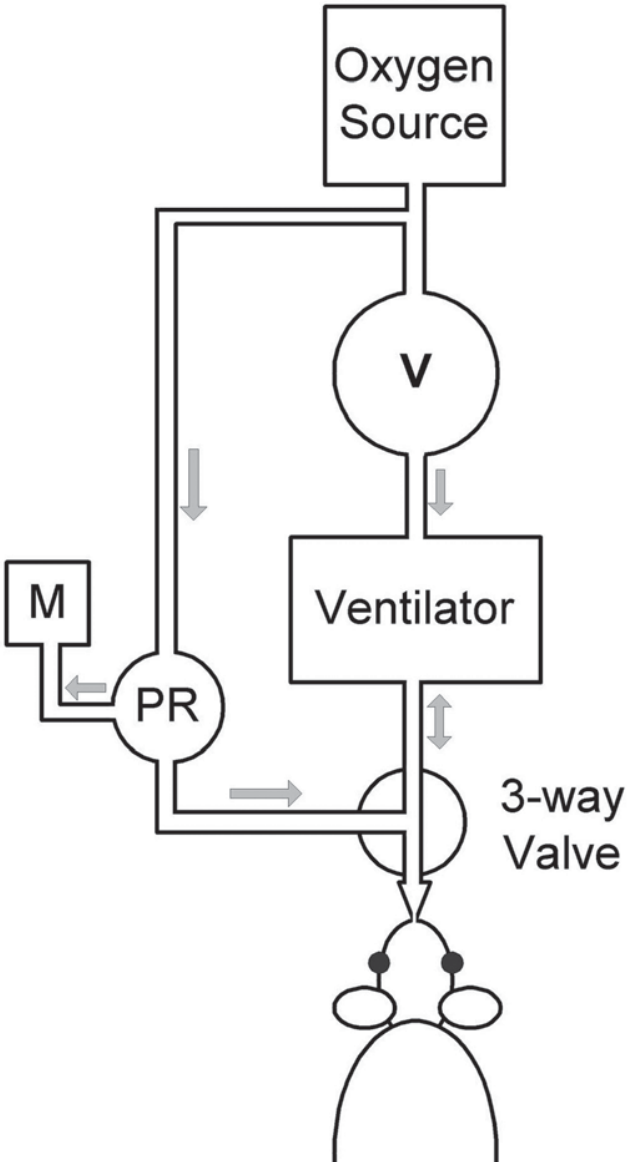

Figure 2. Schematic illustration of the breath-hold system, which switches the animal between the mechanically ventilated system and the constant-pressure breath-hold system. V, vaporizer; M, manometer; PR, pressure reducer.

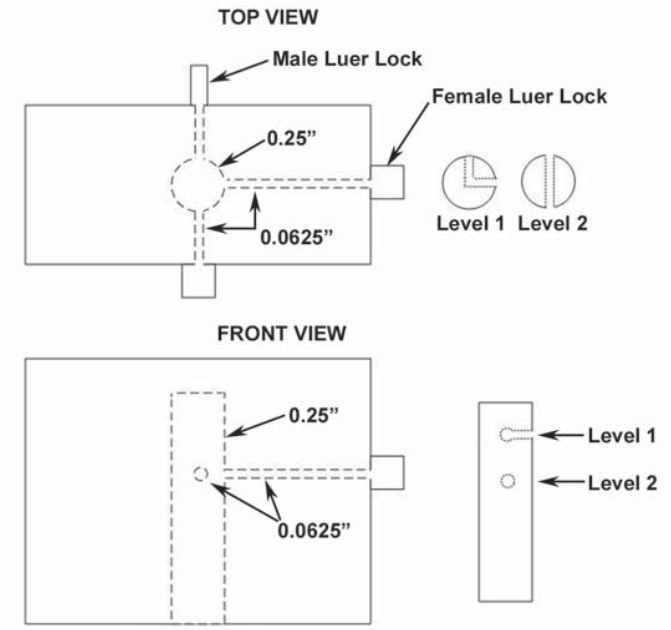

Figure 3. Schematic illustration of the custom-designed electronic 3-way valve, which switches the animal between the mechanically ventilated system and the constant-pressure breath-hold system. Top and front views of the acrylic body of the 3-way valve are on the left. The endotracheal tube of animal is attached to the male Luer lock connector, while the lines from the ventilator and constant pressure oxygen source are connected to the front and side female Luer lock connectors, respectively. Dashed lines represent holes drilled in the block and valve stem. Top and side views of the valve stem which fits in the 0.25-in. hole drilled in the acrylic valve body are present to the right of the illustrations of the valve body. Cross-sectional views of the valve stem at level 1 (path connecting ventilator to animal) and level 2 (path connecting constant pressure oxygen to the animal) are illustrated.

animal, we always tested it with a small balloon phantom that simulates a rodent's lungs, to ensure the system was operating correctly.

With this new device, when the animal is being mechanically ventilated, the valve is turned so that the animal is connected to the ventilator and the line from the pressure-regulated oxygen supply is shut off. To initiate a breath-hold, one manually turns the valve to shut off the ventilator and open the system to the constant-pressure oxygen source. When imaging is complete, the valve is turned manually to switch the animal back to the ventilator.

An electronically activated system subsequently was developed and consists of the same manometer and air-pressure regulator. The manual 3-way valve was replaced with a custom-designed electronically actuated valve (fabricated for us by our Physics Shop). When the solenoid was triggered electronically, it moved the valve stem to switch the animal from the ventilator to the constant-pressure oxygen source. The valve body was constructed of an acrylic block. Two 0.0625-in. holes were drilled into the acrylic block perpendicular to each other. One hole was drilled completely through the block from front to back in order to connect the animal to the ventilator, and the other was drilled from the side of the acrylic block half-way through the block intersecting with the 1st hole in the middle of the block in order to connect the animal with the constant-pressure oxygen source. Two female Luer lock connectors were inserted and glued for connections to the ventilator and breath-hold system. A male Luer lock connector was inserted and glued to connect to the animal's endotracheal tube. A 0.25-in. hole was drilled from the bottom of the block up through the intersection of the 2 previously drilled 0.0625-in. holes for a tetrafluroethylene valve stem. Three 0.0625 in. holes were drilled in the tetrafluroethylene valve stem. One hole was drilled completely through the valve matching the tract drilled completely through the acrylic valve body. The 2nd and 3rd holes were drilled perpendicular to each other and intersect 10 mm above the 1st hole. These 2 holes match the tracts drilled from the breath-hold system to the animal (Figure 3). The actuated valve stem is connected to a pull-type tubular electrical solenoid (Magnetic Sensor Systems, Van Nuys, CA). When the solenoid is not activated, a spring at the base of the solenoid keeps the valve stem in its relaxed extended position, allowing the animal to be maintained on the ventilator. When electric current is applied via a hand-held push button or foot pedal, the solenoid pick-and-hold driver module (SDM840, Magnetic Sensor Systems, Van Nuys, CA) triggers the solenoid and retracts the valve stem 10 mm downward. When retracted, the valve stem closes off the ventilator system and aligns the

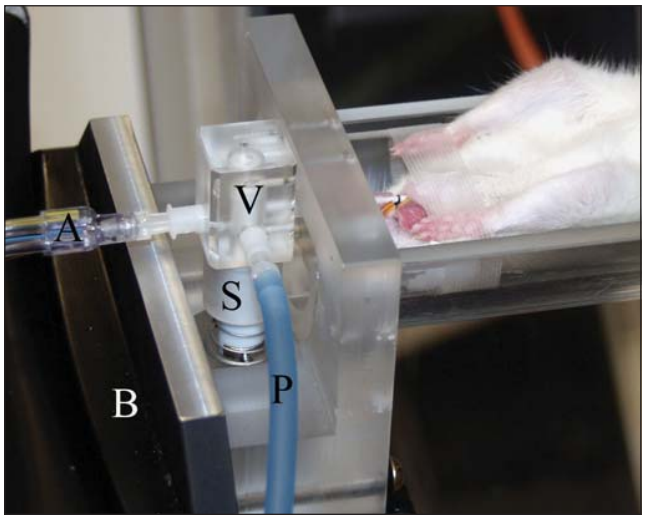

Figure 4. Intubated rat connected to the electronically activated valve (V) of the breath-hold device. Solenoid (S) triggers the Teflon valve to switch the animal from the ventilator (A) to the constant-pressure air source (P). A bracket (B) mounts the electronically activated valve and the animal-positioning device to the bed of the computed tomographic scanner.

holes in the valve stem with the holes in the acrylic body, thereby connecting the animal to the constant-pressure oxygen source. The constant pressure oxygen inflates the lungs and causes an inspirational breath-hold of the animal. Releasing the push button or foot pedal allows the spring to return the solenoid to its relaxed extended position, closing off the breath-hold system and returning the animal to the ventilator system. The valve body, solenoid, and animal holder were attached to the bed of the scanner with an L-shaped piece of acrylic (Figure 4).

Results and Discussion

The prototypic manually operated system provided the desired minimization of motion artifact and resultant improvements in image quality (Figures 5 and 6). However, the necessity for the valve to be in close proximity to the animal and thus to the radiation field of the flat-panel CT scanner during imaging resulted in undesired exposure of personnel operating the valve to radiation during the imaging procedure. In addition, it demanded both hands of a technician to carefully work the valve and required a 2nd technician to be present if any additional animal handling was necessary during the scan acquisition, such as imaging sessions requiring intravenous

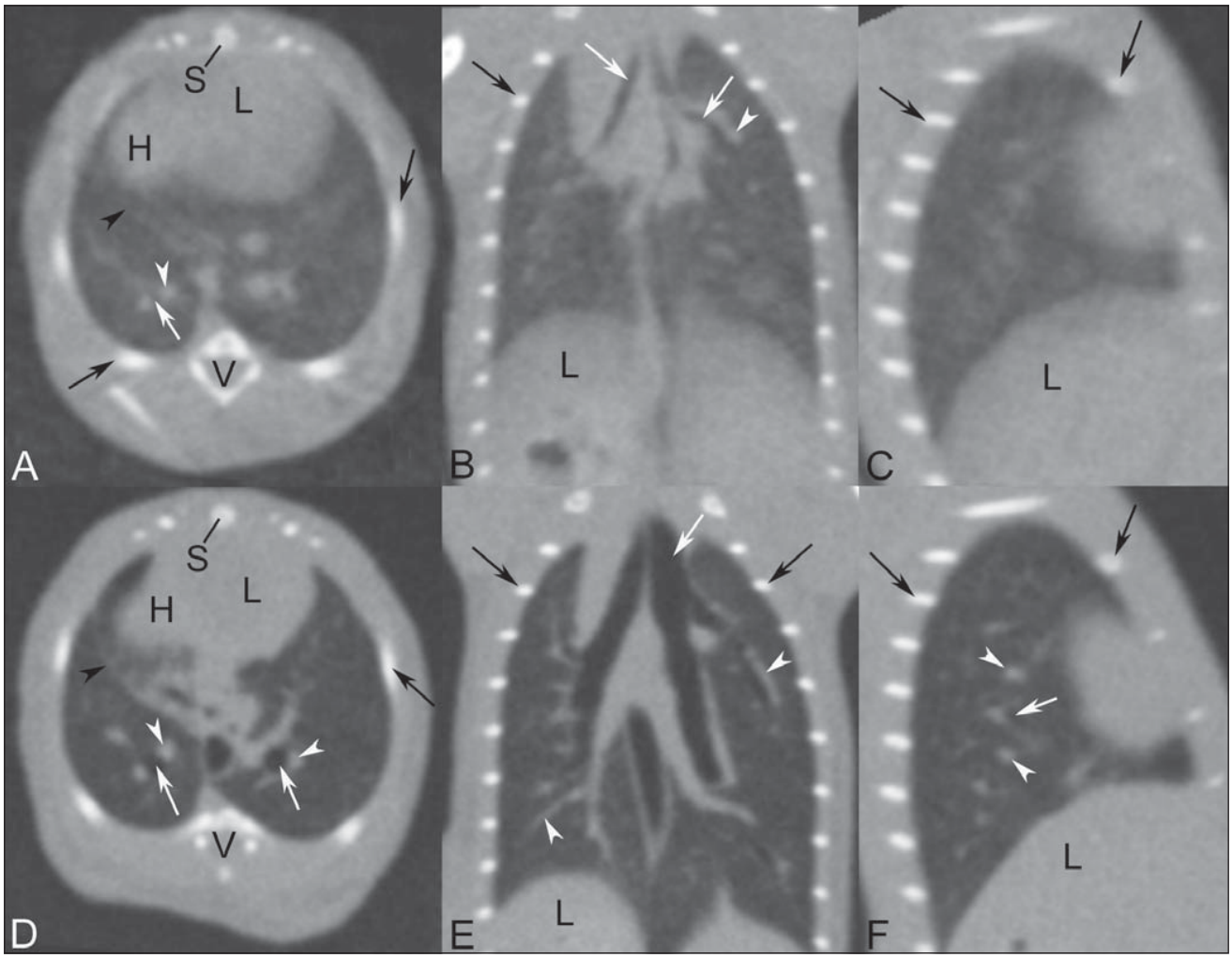

Figure 5. Axial (A, D), coronal (B, E), and sagittal (C, F) images of the thorax of the same 25-g mouse breathing freely during imaging (A through C) and while using a breath-hold of 16 cm of H_2O pressure during imaging (D through F). Note the sharper margins and increased detail of structures in the breath-hold images. Also note the increased visibility of the margins of the individual lung lobe (black arrow head) in the breath-hold images. V, vertebra; H, heart; L, liver; S, sternum; black arrow, rib; white arrow, airway; and white arrowhead, blood vessel. Image voxel size, 100 µm isotropic. All images approximately the same magnification.

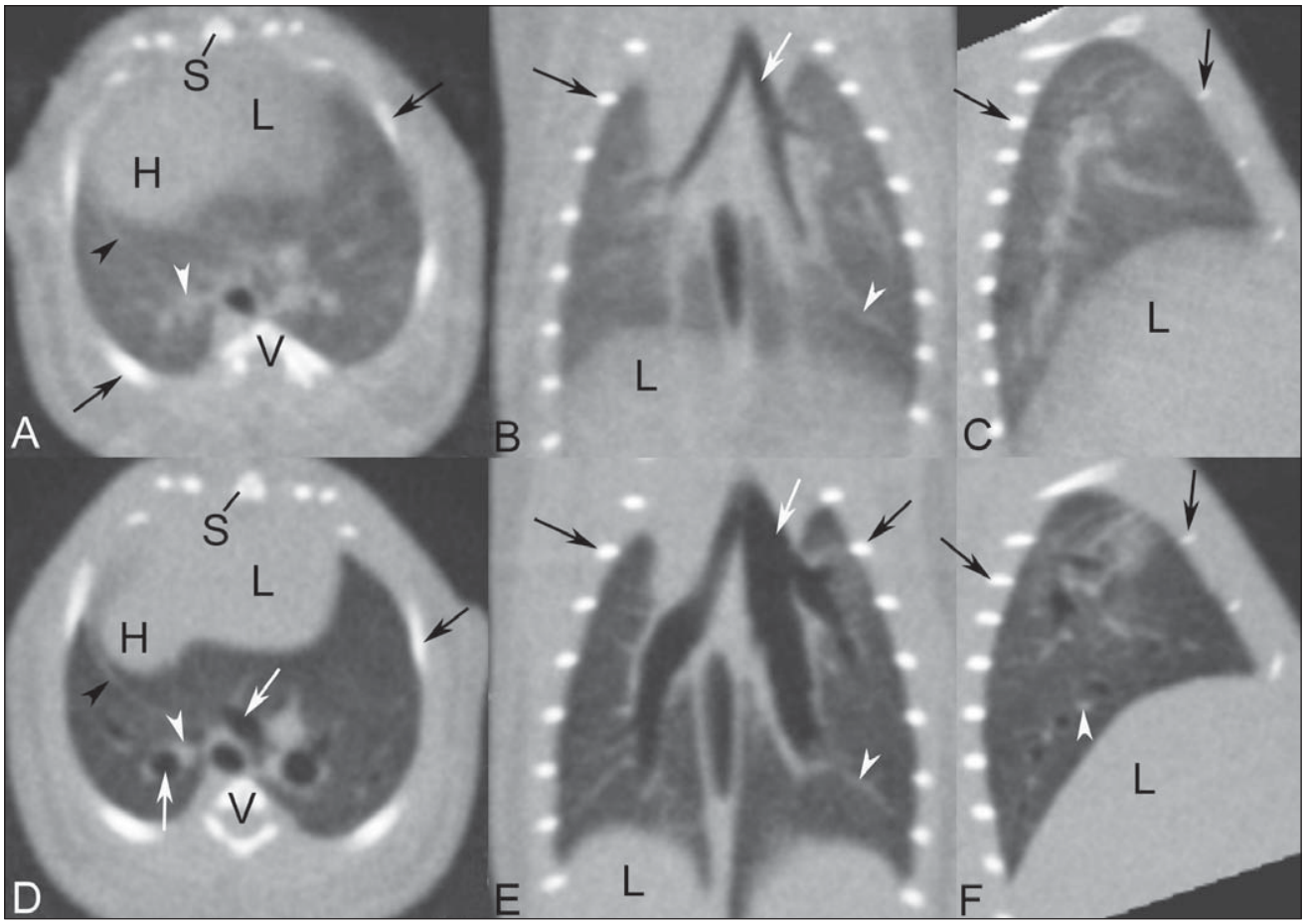

Figure 6. Axial (A, D), coronal (B, E), and sagittal (C, F) images of the thorax of the same 170-g rat breathing freely during imaging (A through C) and while using an 18 cm of H_2O pressure breath-hold during imaging (D through F). Note the sharper margins and increased detail of structures in the breath-hold images. Also note the increased visibility of the margins of the individual lung lobe (black arrow head) in the breath-hold images. V, vertebra; H, heart; L, liver; S, sternum; black arrow, rib; white arrow, airway; and white arrowhead, blood vessel. Image voxel size, 100 µm isotropic. All images approximately the same magnification.

contrast administration. To overcome these issues and to allow our technicians to position themselves further from the radiation source, we designed the electronically activated breath-hold system, which allowed remote activation of a custom designed 3-way valve.

The advantage of this modification is that the solenoid can manually be triggered several feet away from the gantry of the scanner by use of either a hand-held push button or a foot-operated pedal, thus decreasing operator exposure to radiation. Use of the foot-operated switch also frees the hands of the animal technician to perform intravenous administration of contrast during image acquisition (Figure 7).

Multiple mice and rats tested on the manual or electronic version of the breath-hold system recovered without incident. For testing of this device, the acquisitions used 8-s rotation times in mice and rats. Gross and histopathologic examination of selected animals by a veterinary pathologist revealed no pathologic lesions related to the intubation or breath-hold procedures. Ramirez and colleagues[7] have described oxygen saturation levels during 10, 20, and 30 s breath-hold protocols with pure oxygen and have shown that oxygen saturation did not drop below 95%.

There are multiple advantages to the use of constant pressure rather than a calculated volume to inflate lungs during imaging. Ensuring a complete seal of the airway when an uncuffed tetrafluroethylene catheter is used as an endotracheal tube in rodents is difficult, and as a result, leakage is always possible. If there were an incomplete seal around the endotracheal tube, constant-volume inflation of the lungs would allow leakage of the air used to inflate the lungs, and the lungs subsequently would collapse. However, the use of a constant-pressure system instantaneously compensates for air loss from any part of the system and keeps the lungs inflated with a nearly constant pressure. The use of constant pressure eliminates the need to calculate and adjust the volume of air used to inflate the lungs, thus allowing the lungs of animals of different sizes to be inflated to the same extent, which minimizes variations in the amount of lung expansion in studies involving multiple animals. Consistent expansion of the lungs both between animals and between images of the same animal at different time points would help to minimize variations in measurements when using image analysis to perform lung volume or density calculations. In addition, the constant-pressure approach is considered to be safer for the animals than is a constant-volume approach, particularly when the functional lung volume has been reduced as a result of tumor growth, alveolar fibrosis, inflammation, and so on.

The electronically activated system operated as expected. Future plans include fully automating this new breath-hold system by incorporating its control into the software program of the flat-panel CT scanner. This modification will enable the scanner to trigger the breath-hold at the precise moment imaging be-

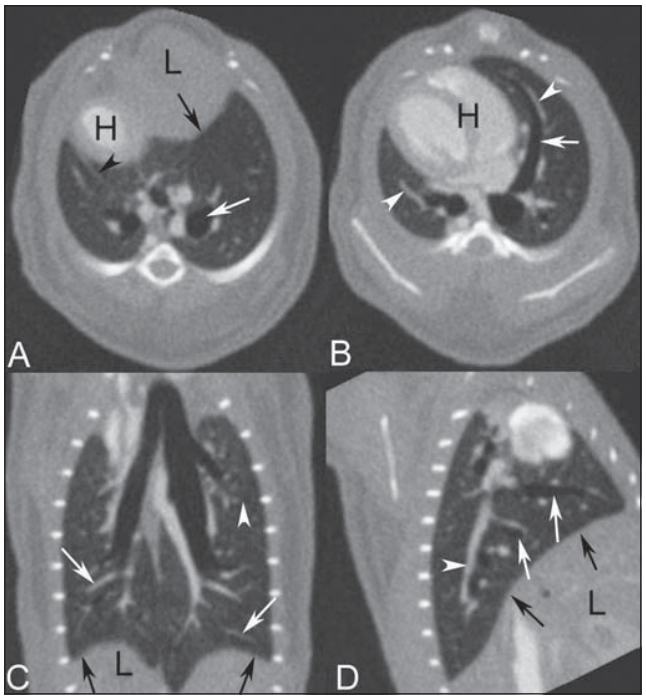

Figure 7. Axial (A, B), coronal (C), and sagittal (D) of thorax of a 25-g mouse during 16-cm H_2O pressure breath-hold imaging with intravenous contrast administration. Note sharp margins of diaphragmatic margin of the lung (black arrows) and increased visibility of the margins of the individual lung lobe (black arrowhead) in the breath-hold images. Chambers of the heart can be visualized as a result of increased signal intensity due to intravenous contrast material present in blood. H, heart; L, liver; white arrow, airway; and white arrowhead, blood vessel. Image voxel size, 100 µm isotropic. All images approximately the same magnification. Image provided courtesy of Dawn Cavanaugh and Elizabeth Travis (University of Texas MD Anderson Cancer Center, Houston, TX).

gins and to discontinue it immediately after image acquisition, thus allowing the shortest possible breath-hold for the animal, thereby minimizing exposure of animal technicians to radiation and freeing them to monitor and perform other functions during the imaging procedure. Although we developed it for this particular preclinical scanner, this technique could also be used when examining rodents on current clinical CT scanners or on fast commercially available similar scanners.

In conclusion, this new electronic breath-hold device is simple and reliable. It has improved the overall quality of the images acquired with this instrument by minimizing motion artifacts. Because anesthetized free-breathing rodents spend the majority of the breathing cycle in expiration with collapsed lungs, a breath-hold which holds the lungs in full inspiration during image acquisition increases tissue contrast in aerated portions of the lung that are adjacent to areas of increased tissue density. Areas of increased tissue density caused by inflammation, fibrosis, atelectasis, or tumors in the aerated portions of the lungs are thus more sharply delineated and easier to identify. Constant-pressure inflation of the lungs also expands the airways and allows easier identification of luminal obstructions and abnormalities. The use of constant pressure also has led to improved, more consistent image analysis of groups of animals because the aerated portions of the lungs of all animals at all points in a study are expanded to the same extent, allowing truer comparisons between imaging time points.

Acknowledgments

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