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A Portable Ventilator with Integrated Physiologic Monitoring for Hyperpolarized ^{129}Xe MRI in Rodents

Rohan S Virgincar^a, Jerry Dahlke^b, Scott H Robertson^c, Nathann Morand^d, Yi Qi^b, Simone Degan^b, Bastiaan Driehuys^{a,b,c}, John Nouls^b

^aBiomedical Engineering, Duke University, Durham, NC, USA ^bRadiology, Duke University Medical Center, Durham, NC, USA ^cMedical Physics Graduate Program, Duke University, Durham, NC, USA ^dEcole des Métiers, Fribourg, Switzerland

Abstract

Hyperpolarized (HP) ^{129}Xe MRI is emerging as a powerful, non-invasive method to image lung function and is beginning to find clinical application across a range of conditions. As clinical implementation progresses, it becomes important to translate back to well-defined animal models, where novel disease signatures can be characterized longitudinally and validated against histology. To date, preclinical ^{129}Xe MRI has been limited to only a few sites worldwide with 2D imaging that is not generally sufficient to fully capture the heterogeneity of lung disease. To address these limitations and facilitate broader dissemination, we report on a compact and portable HP gas ventilator that integrates all the gas-delivery and physiologic monitoring capabilities required for high-resolution 3D hyperpolarized ^{129}Xe imaging. This ventilator is MR- and HP-gas compatible, driven by inexpensive microcontrollers and open source code, and allows for precise control of the tidal volume and breathing cycle in perorally intubated mice and rats. We use the system to demonstrate data acquisition over multiple breath-holds, during which lung motion is suspended to enable high-resolution 3D imaging of gas-phase and dissolved-phase ^{129}Xe in the lungs. We demonstrate the portability and versatility of the ventilator by imaging a mouse model of lung cancer longitudinally at 2-Tesla, and a healthy rat at 7 T. We also report the detection of subtle spectroscopic fluctuations in phase with the heart rate, superimposed onto larger variations stemming from the respiratory cycle. This ventilator was developed to facilitate duplication and gain broad adoption to accelerate preclinical ^{129}Xe MRI research.

Keywords

Hyperpolarized ^{129}Xe ; MRI; ventilator; preclinical; lung; rodent

Introduction

Hyperpolarized (HP) ^{129}Xe MRI is increasingly being applied to characterize regional functional impairment across a broad range of lung diseases (1–3). Upon inhalation, this

unique probe quantifies pathophysiology of the airspaces, interstitial tissues, and pulmonary vasculature by exploiting its solubility and large chemical shifts in different micro-environments. This permits simultaneous, three-dimensional assessment of ^{129}Xe ventilation, barrier-tissue uptake and red blood cell (RBC) transfer (4). As clinical implementation progresses and new disease signatures are identified, it has become increasingly important to test these methods in well-controlled animal models (5–7). Preclinical studies enable longitudinal assessment of disease progression and therapeutic response, with animals serving as their own controls, or terminal studies where imaging findings can be readily compared to histology. Beyond these uses, the preclinical environment provides a platform where novel HP ^{129}Xe imaging and spectroscopy methods can be rapidly developed and validated.

Despite these advantages, the capability to conduct preclinical ^{129}Xe MRI has been limited to only a few centers worldwide (8–12). This stems from the technical challenges and animal handling requirements that are unique to HP gases. These agents must be precisely administered to an uncooperative animal, in a way that is MR-compatible and preserves hyperpolarization. Moreover, rodents require very small tidal volumes (~0.2 ml for mice vs. ~500 ml for humans), which challenges both the precision of gas delivery and the spatial resolution that must be achieved to visualize regional changes in lung pathophysiology.

The most straight-forward means to administer HP ^{129}Xe to an animal is to deliver a single bolus via a gas syringe, as has been demonstrated by *ex vivo* investigations of excised rat lungs (13), and *in vivo* imaging studies of anesthetized rabbits (14). Although this method allows precise control of the volume delivered and permits imaging during suspended lung motion, the relatively small volume of hyperpolarized xenon inherently limits the spatial resolution achievable (a single 2D slice in this case). This challenge becomes even more severe in rats and mice, where the tidal volume is smaller (~2 ml for rats, and ~0.2 ml for mice) and spatial resolution requirements are greater.

In order to provide sufficient magnetization for high-resolution MRI in rodents, it becomes necessary to administer multiple tidal volumes of HP gas, and to acquire image data over multiple breathing cycles. One such approach is to deliver HP gas continuously to spontaneously-breathing, anesthetized mice (8,15). Although this approach has been effectively employed in a number of important studies, the method can be inefficient in its use of HP gas and be affected by irregular breathing patterns of varying depth that cause motion artifacts or limit resolution. Moreover, such acquisitions have been limited to 2D imaging with an in-plane resolution of $0.625 \times 0.625 \text{ mm}^2$ that may obscure some ventilation defects that can be as small as $1.4 \pm 0.3\%$ of the lung volume (16). Resolving such lesions requires high-resolution 3D encoding. This, in turn, requires delivering sufficient HP gas magnetization and mitigating respiratory motion artifacts that would otherwise obscure subtle defects. These requirements are most readily met by mechanically ventilating the animal and performing full three-dimensional encoding, incrementally, over multiple breathing cycles, during brief periods of suspended respiratory motion.

The administration of HP gas to rodents adds unique design requirements that are not met by commercially available MR-compatible ventilators. First, HP gas must be handled using

only materials that contain no ferrous impurities (17). This eliminates most common gas handling materials such as brass and stainless steel. Second, HP gas is readily depolarized by paramagnetic oxygen (18), thus requiring that the two gases be mixed as late as possible in the gas delivery process. Furthermore, given the need to accurately administer very small volumes of compressible gas (~0.2 ml for a mouse), the internal volume of gas delivery components must be strictly limited. And finally, these requirements must all be met without occupying significant space within the close-fitting RF coil housing the animal, nor impairing RF loading. Thus, gas administration must be achieved using a miniaturized, non-conductive, integrated mixing system residing close to animal's trachea (19,20).

In addition to delivering small volumes of HP gas accurately, it is equally important to do so consistently over the entire course of image acquisition. This requires overriding spontaneous breathing by anesthetics that depress the respiratory system and then ventilating the animal mechanically. To perform these procedures safely and ensure recovery after extubation, the animal's physiology (airway pressure, blood oxygen saturation, heart rate, and body temperature) must be continuously monitored and maintained within the desired range. This is particularly important for ^{129}Xe spectroscopy, where line shapes and chemical shifts can be sensitive to changes in pulmonary perfusion and gas-exchange (21). Hence, physiologic monitoring is essential for longitudinal imaging, robust quantification, and to reliably compare animal cohorts.

We have previously reported a rodent ventilator (22) that enabled high-resolution, 3D imaging with hyperpolarized ^3He and ^{129}Xe in mice and rats (23–28). However, this system had several shortcomings. First, it required animals to undergo a tracheostomy to establish gas-tight connections to the ventilator, thereby precluding survival studies. Second, it was operated by a stand-alone computer, running proprietary software (LabView, National Instruments, Austin, TX, USA), and relied on expensive and rapidly outdated data-acquisition boards. Third, it did not integrate the necessary physiological monitoring, which therefore had to be provided using several other systems. This not only increased the cost and complexity of the device, but also limited its portability and ease of dissemination to other centers.

In this report, we present a compact and portable ventilator with integrated physiologic monitoring designed for longitudinal, high-resolution ^{129}Xe MRI in rodents. The system architecture has been simplified to operate using a microcontroller board (Arduino) for precise valve timing and scanner triggering, with a Python/QT user interface driven by an inexpensive single-board computer (Raspberry Pi). These control systems, along with the necessary pneumatics and electronics, are now integrated into two compact enclosures that carry gas to the intubated animal via a single flexible tubing bundle. Physiological measurements of airway pressure and heart rate are now integrated into the system and reported continuously to the user, in addition to the volume of oxygen administered to the animal. Using this system, we demonstrate the ability to acquire high resolution HP ^{129}Xe MR images and spectra, while highlighting its portability and ease of dissemination. Importantly, we show the feasibility of peroral intubation to establish non-traumatic gas-tight seals, thereby enabling longitudinal studies, which we illustrate by imaging a lung tumor model at three timepoints.

Methods

System Architecture

The ventilator and physiologic monitoring system consists of three primary subsystems: the *animal bed*, the *life support module*, and the *control module* (Figure 1). The animal bed is custom 3D-printed and supports the anesthetized rodent such that the lungs are positioned at the magnet isocenter. The bed is equipped with MR-compatible physiologic monitoring sensors: a miniaturized pressure transducer (Fujikura AG206–025k, Servoflo Corporation, Lexington, MA, USA) to measure airway pressure, three pediatric electrode pads (Ambu, Columbia, MD, USA) to record ECG, and a rectally inserted fiber optic (Neoptix, Quebec City, Canada) to measure body temperature. The animal is perorally intubated and ventilated through a flexible tubing bundle that also carries the physiologic monitoring signals. The bundle attaches to the *life support module* residing at the entry of the magnet bore, which delivers the prescribed mixture of gases (O₂, N₂, HP gas), controls the breath-hold, and enables passive exhalation using fast-actuating solenoid or pneumatic valves. These valves have a response time of 8 ms for the solenoids and 20 ms for the pneumatic valve. However, since these delays affect both opening and closing equally, they do not alter the delivered gas volumes.

The MR-compatible *life support module* receives HP gas from a *dose bag* inside a pressurized *canister* mounted on top of the enclosure, as well as oxygen and nitrogen from supply cylinders housed remotely. The *life support module* also contains the ECG and pressure measurement circuitry; it is connected via a single 25-pin cable, carrying valve-actuation and physiologic signals, to the *control module*, located next to MRI console. The *control module*, serving as the primary interface for user-interaction, provides both the ability to change the breathing pattern and continuously monitor physiology. At a more detailed level, the non-MR-compatible *control module* contains the power supplies of the system as well as two distinct micro-controllers: a Raspberry Pi and an Arduino. The Raspberry Pi (model B ARMv8 with 1 GB RAM, <http://www.adafruit.com>) is a single-board computer connected to a keyboard and monitor, running a Linux operating system. The Pi provides the interface by which ventilator settings are controlled and physiologic data is displayed. Because it is subject to real-time interrupts of its operating system, the Pi is not suitable for precise timing control. This task is therefore performed independently by the Arduino micro-controller (Mega 2560, <http://www.adafruit.com>), which runs without an operating system, and thereby can reliably generate precisely timed valve-actuation signals, while also triggering MR data acquisition at the desired phase of the respiratory cycle.

The user controls the following ventilation parameters: breathing rate, inspiration duration, breath-hold duration, and the driving pressures of oxygen, nitrogen and HP gas. The combination of valve timing and driving pressure determines the volume of each gas delivered to the animal (hence, the composition and amount of the tidal volume) and fully defines the breathing cycle.

Detailed part lists, schematics, model files for 3D printing, and calibration data can be downloaded from: <https://sites.duke.edu/driehuyslab/resources/>

Gas Delivery and Volume Control

The essential principle of the ventilator is to deliver precise volumes of O₂, N₂ and HP gas by using carefully timed bursts of flow controlled by fast-actuating valves (22). A schematic of this approach, implemented in the *life support module*, is shown in Figure 2.

Oxygen and nitrogen are each supplied from remote cylinders at high pressure (100 psi) to the *life support module*, where they are regulated (model R-7010, Air Logic, Racine, WI, USA) to a precise driving pressure. Each gas is then directed through a high-impedance brass restrictor (O’keefe Controls Co., Trumbull, CT, USA) attached to the inlet of a fast-switching solenoid-actuated valve (model EC-2–12, Clippard, Cincinnati, OH, USA). This generates a well-defined gas flow rate, which delivers the desired bolus of gas, determined by how long the valve remains open. The exact volumes delivered are calibrated based on the driving pressure and inspiration duration.

In order to acquire HP ¹²⁹Xe MRI, the ventilator is designed to switch from *normal mode*, which delivers a mixture of nitrogen and oxygen, to *HP mode*, wherein nitrogen is replaced with an identical volume of HP gas. The hyperpolarized gas bolus is controlled differently than N₂ or O₂ in order to avoid having it interact with depolarizing ferrous materials. First, HP gas is contained within a perfluoropolymer *dose bag* (Tedlar, Jensen Inert Products, Coral Springs, Florida) that is housed within a pressurized *canister*. It flows through a sapphire restrictor (O’keefe Controls Co., Trumbull, CT, USA) and a polytetrafluoroethylene (PTFE) pneumatically-actuated valve (model PV-1–1134, Parker Hannifin, Tucson, AZ, USA). Switching between modes is accomplished by manually flipping a switch on the *control module*.

Gas is transported between the *life support module* and the rodent by three separate, parallel, 1200- mm-long lines that merge within a low-dead-volume *mouthpiece*, which attaches to the endotracheal catheter. During inspiration, O₂ flows in the dedicated *oxygen line* while either nitrogen or HP gas is transported via the *inert gas line*. To initiate a breath-hold, all valves are closed, during which time the ventilator can trigger motion-free image acquisition. Following the breath-hold, the *exhale valve* is opened, allowing the animal to breathe out passively through the *exhale line*. This breathing cycle is illustrated in Figure 3, as it is displayed by the ventilator, along with ECG trace, scanner trigger point, and airway pressures, along with long-term trends for both. Also depicted in this figure are the valve timing diagram and associated dead volumes. Note, the pressure trace depicts an initial small spike that is attributed to a small, but unavoidable “charging volume” between the flow restrictor and gas-delivery valve (22).

A particular challenge in ventilating rodents is that each gas bolus has a volume that is significantly smaller than that of the gas-delivery components. This can alter the tidal volume and its oxygen content, due to the effects of gas compressibility and dead volumes. This is illustrated for the most stringent case of ventilating a 20-g mouse during HP ¹²⁹Xe MRI, where the oxygen and inert gas boluses account for 50 µl and 150 µl, respectively. During inspiration, O₂ and HP gas are delivered by gas lines reaching a peak-inspiratory pressure of ~12 cmH₂O above end-expiratory pressure. Upon exhalation, this excess pressure will be vented, causing a loss of gas that is 1.2% of the inner volume of each line.

Thus, the *oxygen and inert gas lines* (540- μ l inner volume) each waste 6 μ l of their prescribed bolus. Furthermore, the *mouthpiece* contains a dead volume of 20 μ l between the inspiratory gases and the lungs. Within that dead volume, exhaled gas will be re-administered to the animal, thereby changing the composition of the tidal volume by slightly reducing O₂ content. To compensate for this effect, the tidal volume is prescribed to contain 25% O₂, such that the altered tidal volume entering the animal's trachea will be comprised of at least 20% O₂. Table 1 shows the bolus volume, gas constituents, and gas-delivery timing, along with the choice of restrictors, valves, and tubing diameters for mice and rats.

A key design consideration for the *inert gas line* is to minimize surface-induced ¹²⁹Xe relaxation during transit. The ¹²⁹Xe relaxation time T_1 s in tubing is given by $T_1 = V/(K \cdot A) = D/4K$, where D is the inner diameter of the gas-delivery line, and K is the relaxivity constant. This constant can be estimated for high-purity polymers (29) to be $\sim 9 \cdot 10^{-4}$ mm/s, which for a line of 0.76-mm inner-diameter delivers $T_1 \approx 210$ s. For mice, the typical 0.15-ml bolus of HP gas requires 3.6 breathing cycles to flow through the *inert gas line*, which takes 2.7 s and results in a surface-induced polarization loss of $\sim 1\%$. In addition to this relaxation mechanism, once the ¹²⁹Xe is combined with 25% paramagnetic oxygen at atmospheric pressure, this more potent relaxation mechanism results in $T_1, \text{ oxygen} = 8.9$ s (18,30). For this reason, HP gas is not mixed with O₂ until both gases enter the *mouthpiece*, thereby limiting contact to the 140-ms inspiration period, and yielding oxygen-induced depolarization of $\sim 1.6\%$. To limit other causes of surface-induced depolarization, ¹²⁹Xe flow is kept away from the airway pressure transducer by attaching it on the exhale line, 5-cm distal to the mouthpiece.

Ventilating mice versus rats require different tidal volumes, restrictors, and gas lines as seen in Table 1. Thus, in its practical implementation, the *life support module* contains two different sets of valves and gas lines (one for each rodent species). Ventilation of mice or rats is selected by an electrical switch directing the actuation signals to the desired set of valves and the appropriate tubing bundle. Depolarization effects are similar when ventilating rats, with surface-induced depolarization accounting for $\sim 2\%$ magnetization loss and paramagnetic oxygen accounting for $\sim 2.8\%$.

MR Acquisition

To illustrate the portability and versatility of the ventilator across scanner platforms and rodent species, several studies were carried out using both rats and mice on two different scanner platforms: a 2T Oxford Instruments system running a GE EXCITE 12.0 console; and a 7T Bruker Biospec system running Paravision 6.0.1. All procedures were approved by the Duke University Animal Care and Use Committee (protocol A037-17-02).

Animals were anesthetized (pentobarbital 50–70 mg/kg IP, then hourly with $\sim 30\%$ of the initial dose IP), perorally intubated, placed on the animal bed, and mechanically ventilated in *normal mode* using the breathing parameters in Table 1. Physiological monitoring sensors were installed and the animal bed was inserted into the magnet, where a flow of warm air regulated body temperature between 36 and 37 °C. Xenon, isotopically enriched to 85% (Linde AG, Munich, Germany), was polarized to $\sim 20\%$ (Model 9810, Polarean Inc.,

Durham, NC, USA) and dispensed into the *dose bag* inside the *canister*, which was then pressurized to provide the driving pressure for HP gas administration.

HP ^{129}Xe MRI employed a 3D radial acquisition strategy (12,31) because it is tolerant to under-sampling (12), thereby permitting 3D imaging with modest HP gas volumes (150 ml for mice, 400 ml for rats). Moreover, it permits imaging at short echo times to overcome the short $T2^*$ of xenon *in vivo*, which for the blood-dissolved components can be as short as 1.4 ms at 2 T and 0.5 ms at 7 T. The sequence, and 3D k-space sampling pattern are shown in Figure 4, along with representative breath-hold-gated ^{129}Xe raw FIDs. This illustrates that magnetization is depleted by the multiple RF excitations applied during a breath-hold, but, over multiple breaths, remains relatively stable by virtue of repeated replenishment of new ^{129}Xe delivered by the ventilator.

In addition to ^{129}Xe MRI, XH MRI was also acquired for anatomical reference, with the same 3D radial sampling scheme. ^{129}Xe and ^1H MR acquisition parameters are listed in Table 2 for mice at 2 Tesla and rats at 7 Tesla. While gas-phase MRI could be acquired using short, hard RF pulses (and thus short echo times), the $\sim 100\times$ weaker dissolved ^{129}Xe signal at ~ 200 ppm above the gas frequency must be excited using a longer, frequency-selective RF pulse. This is necessary to suppress the much larger gas-phase signal, while still allowing the echo time to remain sufficiently brief to capture the short- $T2^*$ dissolved-phase signal.

^{129}Xe spectroscopy relies on the same RF pulses and gating strategies as imaging, but with longer read-out periods. Dissolved ^{129}Xe spectroscopic data was as acquired at 7 T using a bandwidth of 50 kHz, 512 points and a TR of 19 ms. Complex spectra were fitted to extract the intensity, frequency, linewidth, and phase of the three main ^{129}Xe resonances: gas, barrier tissue, and RBC (32).

Results and Discussion

^{129}Xe MRI Across Scanner Platforms, Field Strength and Species

The utility of the ventilation and physiologic monitoring system was demonstrated at 2 Tesla (12,32) in a longitudinal study of tumor growth in Balb/C mice inoculated with 4T1 cancer cells. Figure 5 shows HP ^{129}Xe gas-phase and dissolved-phase images in a healthy mouse, as well as an overlay of ^{129}Xe and co-registered ^1H anatomical images of a tumor-bearing mouse, scanned at 3 different timepoints after inoculation.

In the healthy mouse, the gas-phase ^{129}Xe images reveal airways down to the 6th generation. In the tumor-bearing mouse, the ventilation images reveal a small defect in the left lung (see Figure 5c), occupying only 0.2 μl , or 0.04% of the lung volume. In all animals, the dissolved-phase ^{129}Xe images depict diffusive ^{129}Xe transfer to the pulmonary interstitial tissues and blood vessels, revealing clear signal in the pulmonary veins, the left ventricle, and the aorta. Both gas- and dissolved-phase images appear free of motion artifacts at an isotropic resolution of 156 μm and 312 μm , with SNR of 14 and 23. This demonstrates that intubation permitted repeatable ventilation and data acquisition over the administration of 600 tidal volumes during image acquisition.

The portability of the system was demonstrated by acquiring gas-phase and dissolved-phase ^{129}Xe MRI on a 7-Tesla platform, while the cross-species adaptability of the system was illustrated by ventilating rats. This transition was achieved by moving the ventilator modules beside the 7-T magnet and switching the control signals towards the valves appropriate for rats. The resulting gas and dissolved-phase ^{129}Xe images from a 200-g healthy Sprague-Dawley rat are shown in Figure 6.

Acquisition of the gas-phase image consumed 180 tidal volumes, each containing 1.5 ml of ^{129}Xe . Inspection of the raw data acquired over the course of the acquisition showed that FID amplitudes varied by less than 4.1% from breath-to-breath (data not shown), which attests to the consistency of mechanical ventilation. The dissolved-phase images demonstrate that 3D radial imaging overcomes the exceedingly short $T2^*$ of dissolved-phase ^{129}Xe at 7 T ($T2^* \approx 0.5$ ms).

Although we have only presented mouse images at 2T and rat images at 7T, it must be noted that the ventilator is capable of being used with either species on either magnet. The choice of rodent species and magnet field strengths reflects practical considerations in demonstrating the versatility of this system.

^{129}Xe spectroscopy

In addition to imaging, ^{129}Xe spectroscopic parameters are highly sensitive to lung inflation and physiological changes such as oxygenation (32, 33). Thus, accurately measuring these spectroscopic features arguably provides the most rigorous test of consistent ventilator performance. To this end, ^{129}Xe spectroscopy was used to investigate the effects of the breathing cycle on ^{129}Xe gas- and dissolved-phase signals. A dissolved-phase ^{129}Xe spectrum was acquired every 19 ms over the course of 50 consecutive breathing cycles, with the three main resonances (gas, barrier tissue, and RBC) shown in Figure 7a. These three resonances were identified at chemical shifts of -0.9 ppm, 196.1 ppm and 209.6 ppm; and linewidths of 145 , 636 , and 419 Hz, respectively. For each resonance, its intensity and chemical shift are plotted over five breaths to demonstrate their evolution during ventilation (Figure 7b).

The ^{129}Xe spectroscopy dynamics revealed temporally repeating patterns of each resonance. Most notable was the change in signal intensities in synchrony with ventilation. The intensity of the gas-phase resonance nearly doubled from end-expiration to full-inspiration. The intensities of the barrier and RBC resonances also followed the ventilation cycle, but with smaller changes of 10% and 15% respectively. This difference between the dissolved vs. gas-phase ^{129}Xe signal dynamics may be attributable to lower capillary blood volumes at peak inspiration (21).

In addition to affecting intensity, the ventilation cycle also caused subtle variation in the chemical shifts of each resonance. Notably, the gas-phase frequency shifted upward by 0.3 ppm at full-inspiration relative to end-expiration. Perhaps more surprisingly, the dissolved phase resonances exhibited small oscillations that were significantly faster than the ventilation rate. In this example, the oscillations occur at a frequency of ~ 5 Hz (corresponding to the animal's heart rate) and are most notable in the chemical shifts of the

dissolved-phase resonances, and to a lesser extent in their amplitudes. Although much work is needed to understand these dynamics in detail, these data illustrate that a potentially new realm of interesting subtle spectroscopic fluctuations can be detected. Ultimately, these may represent additional interesting markers that change with pathophysiological state. However, such features can only be teased out if signals are sufficiently robust from breath-to-breath, as enabled by consistent mechanical ventilation.

Limitations

Although, this ventilator and physiologic monitoring system enables high-resolution ^{129}Xe MRI, it presents several opportunities for further improvement. Firstly, the electrical lines connecting the ECG and airway pressure signals from the bore center to the *life support module* can act as antennas, transmitting ambient electrical noise into the coil, thereby degrading signal-to-noise ratio. To avoid such phenomena, the lines must be shielded and grounded to the *life support module*, which, in turn, must be grounded to the magnet chassis. A future preferred solution may be to convey these signals via non-conductive fiber optics. Secondly, the system does not currently accommodate inhaled anesthetics. Instead, animals must be anesthetized by injectable agents, such as pentobarbital, which are more difficult to administer and maintain in challenged animals over relatively long scan sessions (34). Lastly, the pressure required to ensure that the intubation catheter forms a tight seal has the potential to injure the glottis. In this work, observation of the animals after extubation revealed no signs of labored breathing. Moreover, any trauma caused was not severe enough to prevent repeated intubation on a timescale of days. However, future studies should include a more thorough optical examination of the glottis and surrounding tissue to better characterize the potential occurrence and severity of intubation trauma.

Conclusion

The rodent ventilator with integrated physiological monitoring has demonstrated its utility for acquiring high-resolution 3D images of gas-phase and dissolved-phase hyperpolarized ^{129}Xe in rats and mice, with different magnets and across field strengths. A particularly critical feature is its ability to preserve HP ^{129}Xe polarization while precisely delivering accurately-timed, identical tidal volumes, and triggering MR data acquisition at any phase of the respiratory cycle. At 7 T in a rat, the ventilator is consistent enough to permit the detection of subtle spectroscopic fluctuations in phase with the heart rate, superimposed onto larger variations stemming from the respiratory cycle. The ventilator is compact and portable between magnets or imaging centers. A duplicate of this system was shipped and installed at the Radiobiology Research Institute at Oxford University in the United Kingdom, where it was successfully used to conduct *in vivo* HP ^{129}Xe MRI in mice. Moreover, the system can be used with perorally intubated animals, opening the way to longitudinal studies of disease progression and therapy response.

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Figure 1:

Primary subsystems. At the magnet center, the rodent lies on the animal bed, connected to physiologic monitoring sensors. The intubated animal (the intubation catheter is magnified) is linked to the *life support module*, which resides at the entry of the bore and contains the gas-delivery valves, physiologic monitoring circuitry, and HP gas supply. The *life support module* is connected via a single cable to the *control module*, which is located next to the MRI user console.

Figure 2: Schematic showing the primary gas-delivery components within and connected to the *life support module*. The user interface and the *control module* are also depicted (bottom right).

Figure 3:

a) Tapered intubation catheters for mice (22G, blue) and rats (14G, orange). b) Display of the ventilator while ventilating a rat, showing real-time airway pressure (black), the MR trigger (pink), ECG (blue) with automatic r-wave detection. The evolution of the peak inspiratory and expiratory pressures (green), and heart rate (red) over the duration of the study are also shown. The top right corner of the display shows the current heart rate, ventilation mode, airway pressures, gas-delivery pressures and associated tidal volumes. c) Cross-sectional view of the intubation catheter connected to the *mouthpiece*, showing the merging gas lines, and the dead volume. d) Actuation pattern of the gas delivery valves (drawn in synchrony with the airway pressure trace on the monitor).

Figure 4:

a) Schematic of the short-TE 3D radial imaging sequence. b) Representative 60 radial rays, acquired in randomized order, to sample k-space uniformly over time (illustrated by the first 20 rays being shown in red). c) Representative raw ^{129}Xe gas-phase FIDs from the first three breath-holds of a rat imaging study. Within each breath-hold, 20 FIDs are acquired, with the amplitude of each subsequent FID decreasing due to RF-induced loss of magnetization. However, this is replenished by continued ventilation, and the resulting ^{129}Xe signal intensity remains stable over the course of the acquisition, with only a slow decrease due to T_1 relaxation in the dose bag (d). Note that the time axis in (c) is discontinuous because no data was recorded between breath-holds.

Figure 5:

^{129}Xe MRI in mice at 2 T. a) Coronal and axial slices of gas-phase ^{129}Xe MRI acquired in a healthy mouse with 156- μm isotropic resolution and associated maximum-intensity projections (right). The high-resolution images revealed homogeneous ventilation in the thoracic cavity. b) Dissolved-phase ^{129}Xe images acquired at 312- μm resolution, combining the signal from both the interstitial barrier tissues and blood vessels. These images clearly reveal the left ventricle and aortic arch (white arrows). c) Anatomical overlay of gas-phase ^{129}Xe (top) and dissolved ^{129}Xe (bottom) in the same tumor-bearing mouse 3, 5, and 6 weeks post inoculation. Key features include ventilation defects at week 5 and 6 (white arrows), and a large tumor in the posterior right lung at week 6 accompanied by matched ventilation and gas-exchange defects (yellow arrow) and a loss of ^{129}Xe signal in the left ventricle.

Figure 6:

^{129}Xe MRI in a healthy rat at 7 T. A) Coronal and axial ^{129}Xe gas phase MRI slices with 390- μm isotropic resolution overlaid over ^1H anatomy. B) Coronal and axial slices of ^{129}Xe dissolved-phase MRI with 780- μm isotropic resolution.

Figure 7:

a) Representative ^{129}Xe dissolved-phase spectrum in the rat thorax at 7T (left), and fitting results (right) showing its 3 components (gas, barrier tissue, and RBC). b) The temporal evolution of the intensity and chemical shift of the 3 resonances over 5 representative breaths.

Table 1:

Gas-delivery parameters and components for a 20-g mouse and 200-g rat.

Gas	Tidal volume			Breathing cycle parameters				Trigger ³	Driving Pressure	Flow restrictors		Valves		Tubing		
	Prescribed	Administered	Rate	Inspiration	Breath-hold	Expiration	Material			ID ¹	Wetted material	Actuation	Material	ID ¹	Volume	
	(μl)	(%)	(μl)	(%)	(min -1)	(ms)	(ms)	(ms)	(psi)	(mm)	(mm)	(mm)	(mm)	(mm)	(μl)	
Mouse	O ₂	50	25	39	21				2	Brass	0.04	Steel	Solenoid	PEEK	0.76	540
	N ₂	150	75	129	69				3.4	Brass	0.1	Steel	Solenoid			
	Xe	150	75	129	69	80	140	200	160					PEEK ²	0.76	540
	Exhale	-		20	11					2.1	Sapphire	0.17	Teflon	Pneumatic		
Rat	O ₂	500	25	405	21				1.9	Brass	0.15	Steel	Solenoid	PEEK	1.14	1220
	N ₂	1500	75	1298	67				1.9	Brass	0.26	Steel	Solenoid	Tygon	2.06	4000
	Xe	1500	75	1298	67	60	250	250	270					Tygon ²	2.06	4000
	Exhale	-		240	12					2.3	Sapphire	0.34	Teflon	Pneumatic		
									-	-	-	Steel	Solenoid	Tygon	2.79	7336

¹ The inner diameter of restrictors and tubing are specified in inches by the supplier but have been converted to metric units for consistency

² Nitrogen and HP gas flow through the same *inert gas line*

³ MR trigger position is relative to the start of inspiration.

Table 2:

3D-radial MRI parameters for ^{129}Xe gas, ^{129}Xe dissolved, and XH MRI in mice at 2T, and rats at 7T.

Parameters	Mouse - 2T			Rat - 7T		
	^{129}Xe gas	^{129}Xe dissolved	^1H	^{129}Xe gas	^{129}Xe dissolved	^1H
RF pulse	hard	3-lobe sinc	hard	hard	3-lobe sinc	hard
RF duration (μs)	132	1200	132	100	310	100
Excitation frequency (MHz)	23.647	gas+4.66 kHz	85.54	83.06	gas+17.51 kHz	300.3
TE (ms)	0.384	1.232	0.384	0.056	0.25	0.056
TR (ms)	10	50	8	10	15	10
Flip angle	30°	30°	15°	15°	20°	5°
Bandwidth (kHz)	8.06	15.63	31.25	10	100	100
NEX	1	1	1	1	7	1
Data points per ray	64	32	128	64	64	64
Matrix Size ³	128	128	256	128	128	128
Field of view (mm)	20	40	40	50	100	50
Gating	yes	yes	yes	yes	no	yes
Rays (total)	3001	2901	51471	3582	2073	14328
Rays per breath-hold	5	4	20	20	-	20
Breath-holds (total)	601	726	2574	180	218	717
Scan time	7m 30s	8m 5s	32m 11s	3 m	3m 38s	11m 57s