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Engineering of a Low-Cost Open-Source Hypoxia Chamber for Rodent Models of Obstructive Sleep Apnea

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Abstract

Introduction:

Reproducible intermittent hypoxia (IH) exposure is essential for investigating the pathophysiology of obstructive sleep apnea (OSA) and its cardiopulmonary consequences. However, commercially available IH systems are often prohibitively expensive, technically restrictive, and reliant on proprietary architectures. This study aimed to develop and validate a low-cost, open-source IH chamber capable of automated oxygen cycling, real-time environmental monitoring, and multi-animal capacity for translational sleep-apnea research.

Methods

An open-source IH system was constructed using a Raspberry Pi–based control unit, solenoid-valve gas switching, and custom Python software for automated hypoxia–normoxia cycling and data logging. Chamber-level oxygen concentration (FiO_2) was continuously monitored using a galvanic O_2 sensor, alongside temperature and humidity sensors; a carbon monoxide sensor was included solely as a qualitative safety indicator. Two validated IH regimens were tested: moderate IH (10–12% O_2; 20–30 cycles·h^{-1}) and severe IH (5–7% O_2; 30–60 cycles·h^{-1}). Precision, reproducibility, and environmental stability were assessed using coefficient of variation analysis and one-way ANOVA ($p < 0.05$).

Results

The chamber reliably achieved rapid and reproducible FiO_2 oscillations between normoxia (21%) and target hypoxic nadirs (10–12% or 5–7%) within 20–25 s, with cycle-to-cycle variability < 5%. Environmental conditions remained stable during prolonged exposures (22–24°C; 55–80% relative humidity), and effective chamber ventilation prevented abnormal gas accumulation. The total system cost was approximately SAR 2,990 (≈ US$800), representing < 5% of the cost of a commercial OxyCycler while delivering comparable desaturation dynamics.

Conclusion

This validated, low-cost, open-source IH chamber provides a scalable and reproducible platform for modeling OSA-related intermittent hypoxia in rodents. Its affordability, transparency, and multi-animal capacity support broader adoption and standardization of IH research across laboratories worldwide.

1. Introduction

Obstructive sleep apnea (OSA) is one of the most prevalent sleep-related breathing disorders worldwide and is characterized by recurrent episodes of partial or complete upper airway collapse during sleep,

leading to intermittent hypoxemia, hypercapnia, intrathoracic pressure swings, and sleep fragmentation [1,2]. Epidemiological studies estimate that moderate-to-severe OSA affects nearly one billion adults globally, with prevalence increasing in parallel with obesity, aging, and cardiometabolic disease burden [3,4]. A defining physiological hallmark of OSA is intermittent hypoxia (IH), arising from repetitive cycles of oxygen desaturation and reoxygenation secondary to obstructive respiratory events [2,5]. Importantly, IH is a consequence of OSA pathophysiology, not a causal factor in the development of the disorder. These repetitive hypoxia–reoxygenation cycles trigger oxidative stress, sympathetic activation, systemic inflammation, endothelial dysfunction, and metabolic dysregulation, which together contribute to the development of OSA-associated comorbidities, including hypertension, atrial fibrillation, stroke, insulin resistance, cognitive impairment, and increased all-cause mortality [6–9].

Rodent Intermittent Hypoxia Models and Their Physiological Scope

Over the past several decades, rodent IH models have played a central role in advancing mechanistic understanding of OSA-related cardiovascular, metabolic, and neurocognitive consequences by exposing animals to precisely controlled hypoxia–normoxia cycles, which isolate the hypoxic stimulus and enable investigation of downstream molecular and physiological responses under highly reproducible conditions [10–12]. However, it is critical to distinguish rodent IH exposure from human OSA physiology. While IH models faithfully reproduce the hypoxia–reoxygenation component of OSA, they do not recapitulate upper airway obstruction, increased respiratory effort, sleep fragmentation, arousal responses, or carbon dioxide retention that occur in patients with OSA [13–15]. Consequently, IH models should be viewed as reductionist experimental tools designed to study the biological effects of intermittent hypoxemia rather than comprehensive surrogates of the full OSA syndrome. Recognizing these limitations is essential for appropriate interpretation and translational relevance of IH-based findings.

Evolution of Hypoxia Chamber Systems

Early IH exposure systems consisted of small, fully enclosed animal chambers with manually adjusted gas inflow rates. While these designs provided initial proof-of-concept for hypoxia research, they were limited by slow gas exchange kinetics, imprecise oxygen control, and poor reproducibility, particularly for short hypoxic cycles relevant to rodent models [16,17].

The introduction of automated hypoxia systems—most notably computer-controlled platforms such as the OxyCycler (BioSpherix, USA)—represented a major technical advance by enabling programmable FiO_2 cycling through solenoid-regulated gas delivery [18]. These systems allow highly reproducible desaturation profiles and precise protocol control but are associated with substantial financial cost, often exceeding USD 40,000, and rely on proprietary hardware and closed-source software architectures [19]. As a result, their use remains largely confined to well-funded laboratories, limiting accessibility and scalability across institutions.

In addition, many commercial platforms are optimized for single-animal exposure, which constrains experimental throughput and complicates studies requiring multiple exposure groups, pharmacological interventions, or longitudinal designs [20].

To address cost and accessibility barriers, several groups have developed custom hypoxia chambers using plexiglass enclosures with manually or semi-automated gas regulation. Although more affordable, such systems often lack closed-loop control and exhibit delayed gas exchange, limiting their ability to reproduce rapid hypoxia–reoxygenation cycles typical of rodent IH protocols (e.g., 20–30 s nadirs) [20,21]. These constraints may lead to attenuated or inconsistent oxygen profiles, reducing experimental reproducibility.

Another limitation of many legacy and custom designs is the **restricted scope of environmental monitoring**. Oxygen concentration is frequently the sole parameter measured, whereas carbon dioxide accumulation, temperature, and humidity are often unmonitored despite their independent influence on respiratory drive, metabolic rate, and airway physiology [22–24]. Insufficient environmental monitoring complicates cross-laboratory comparisons and introduces uncontrolled variability.

While recent open-source IH chamber designs using Arduino or Raspberry Pi controllers have been described [25,26], most remain limited by single-animal capacity, incomplete environmental sensing, or lack of detailed validation and reproducibility documentation.

Together, these limitations underscore the need for an **affordable, scalable, and transparent IH chamber system** that combines precise oxygen control with comprehensive environmental monitoring and multi-animal capacity. Advances in open-source electronics, including low-cost single-board computers, modular sensors, and open programming frameworks, now enable the development of sophisticated laboratory systems at a fraction of the cost of commercial alternatives [27].

Open-source biomedical engineering solutions have already demonstrated feasibility and reliability across a range of applications, including ventilators, infusion systems, and environmental monitoring platforms [26,27]. Applying similar principles to hypoxia research offers the opportunity to enhance accessibility, reproducibility, and methodological transparency.

Study Objectives

In the present study, we designed and validated a **low-cost, open-source intermittent hypoxia chamber for rodent models** that addresses key limitations of existing systems. Specifically, the objectives were to:

1. **Increase experimental throughput** by constructing a chamber capable of housing up to 10 rats simultaneously.
2. **Enhance usability and transparency** through a real-time, user-friendly monitoring interface for chamber environmental conditions.

3. **Improve environmental control and reproducibility** by continuously monitoring oxygen concentration, temperature, and humidity during programmable hypoxia–normoxia cycling.

While the system is optimized for modeling the intermittent hypoxia component of OSA, its modular design also enables application to other experimental paradigms, including ischemia–reperfusion injury, chronic respiratory disease models, and high-altitude physiology research.

2. System Design and Methods

2.1 Study Design

This study employed a controlled experimental design using a rat model to investigate the effects of intermittent hypoxia (IH) exposure. The experimental paradigm was designed to reproduce the **hypoxia–reoxygenation component** of obstructive sleep apnea (OSA), which is widely used in preclinical research to study downstream cardiovascular, pulmonary, and systemic consequences of OSA-associated hypoxemia [28].

It is important to emphasize that IH is a **reductionist model** that isolates cyclic hypoxemia and does not fully recapitulate key features of human OSA such as upper airway obstruction, sleep fragmentation, ventilatory effort swings, or CO2 retention. Nevertheless, IH models are extensively validated and remain among the most widely used approaches for investigating mechanisms of OSA-related hypoxic stress in cardiopulmonary systems [29]. All experimental procedures were conducted according to predefined protocols with tightly controlled environmental and exposure parameters to ensure reproducibility and minimize variability.

2.1.1 Study Area and Experimental Setting

The study was conducted between April 1 and July 29, 2025, at the Animal Experimental Center of King Saud University (KSU), Riyadh, Saudi Arabia. All procedures were performed in accordance with internationally accepted standards for laboratory animal care and use, including the **NIH Guide for the Care and Use of Laboratory Animals** and the **ARRIVE 2.0 guidelines** [30]. Animals were housed under ambient room air conditions, with temperature maintained at 22–24°C and relative humidity at 40–60%, under a 12:12 h light–dark cycle (lights on at 07:00) [31]. Rats were provided ad libitum access to standard laboratory chow and water. Prior to experimental interventions, all animals underwent a 4-day acclimatization period.

2.1.2 Animal Selection and Rationale

A total of fifty (50) adult male Sprague–Dawley rats, weighing 300–400 g, were included in the study. This strain was selected due to its well-characterized physiology, docile temperament, and widespread use in cardiopulmonary research, making it suitable for controlled IH exposure, physiological monitoring, blood sampling, and tissue collection **[32]**.

Rats share substantial genetic and physiological similarities with humans, including high conservation of protein-coding genes and comparable cardiopulmonary structural and functional characteristics, supporting their translational relevance for hypoxia-related cardiopulmonary injury studies **[33]**. Only clinically healthy animals free from overt respiratory or cardiovascular abnormalities were included following routine veterinary assessment.

2.1 Control Unit

The control system was built around a Raspberry Pi 4 Model B (Raspberry Pi Foundation, UK) featuring a quad-core 1.5 GHz ARM Cortex-A72 CPU and 4 GB RAM [34]. The system operates on Raspberry Pi OS (Linux-based) and executes custom Python 3.9 scripts to control hypoxia–normoxia cycling, valve actuation, and sensor data acquisition. System control and real-time data visualization were provided via a 5-inch capacitive touchscreen **[35]**.

High-current devices (solenoid valves) were actuated via a dual-channel 5 V relay module, electrically isolated from Raspberry Pi GPIO outputs (Fig. 1).

2.2 Gas Delivery System

FiO_2 was regulated using electrically actuated solenoid valves connected to compressed gas sources. Two 12 V DC solenoid valves (Model YS-12, Ningbo King Shengda Solenoid Tech., China) controlled nitrogen inflow and room air/oxygen inflow **[36]**. Gas sources were supplied via medical-grade cylinders equipped with precision pressure regulators and flowmeters (Aalborg Instruments, USA) **[37]**. A continuous background airflow (~ 1–2 L/min) was maintained via a needle valve to prevent accumulation of expired gases, consistent with established IH protocols **[38]**. The timing-based on–off solenoid switching strategy is consistent with widely used IH systems employing either timers or sensor-based control [39].

2.3 Environmental Sensors

Chamber FiO_2 was monitored using an electrochemical oxygen sensor module (Gravity I²C O_2 Sensor, DFRobot, China) incorporating a galvanic cell (Teledyne R-17A type) [40]. Calibration was performed using certified gas mixtures (5%, 10%, and 21% O_2; balance N_2) [41].

An MQ-7 semiconductor sensor (Hanwei Electronics, China) was included **solely** as a qualitative carbon monoxide (CO) safety indicator and was not used for CO_2 measurement **[42]**. Temperature and humidity were monitored using a BME280 sensor (Bosch Sensortec, Germany). Analog signals were digitized using an ADS1115 16-bit ADC where required.

2.4 Sensor Calibration and Verification

The chamber O_2 sensor (Gravity I²C O_2 sensor; galvanic cell) was calibrated prior to experimental runs using certified reference gases (21% O_2, 10% O_2, and 5% O_2; balance N_2). The chamber was flushed with each reference gas until the O_2 reading stabilized, and the corresponding raw sensor outputs were

recorded to generate a calibration curve converting sensor output to %FiO_2. Calibration was repeated at regular intervals (e.g., weekly) and whenever the sensor was replaced.

Temperature and humidity measurements (BME280) were verified before experiments by comparison to an external reference thermometer/hygrometer under stable ambient conditions (22–24°C; 40–60% RH). Agreement within the manufacturer-stated accuracy (± 1°C; ±3% RH) was required prior to data collection. All calibration and verification steps were performed to ensure accuracy and reproducibility of chamber environmental monitoring.

2.5 Hypoxia Chamber Construction

The hypoxia chamber was a custom-built rectangular enclosure fabricated from translucent Plexiglas sheets (100 cm × 40 cm × 60 cm), corresponding to an internal volume of approximately **240 L**, which supports multi-rat housing while maintaining efficient gas exchange kinetics **[43]**. The chamber was constructed in the Biomedical Engineering Workshop at King Saud University. To ensure airtight integrity, all panel joints and seams were sealed using silicone gasket material, and animal access was provided via a tight-fitting Plexiglas lid incorporating a rubber gasket to maintain a secure seal.

Two bulkhead ports were installed to connect the gas delivery tube to a dedicated inlet and outlet. The inlet port was positioned to direct incoming gas toward the central chamber space to promote mixing, while the outlet port was placed near the upper region of the chamber to facilitate effective flushing and venting via an exhaust line **[44]**. To reduce visual stress during exposures, reflective one-way film was applied to selected interior surfaces, enabling external observation while limiting animals' perception of surrounding movement. The chamber included a detachable stainless-steel raised mesh floor positioned a few centimeters above the base to support the animals, improve gas diffusion, and separate waste; the mesh floor and bedding were removable for cleaning and sterilization.

Before each experimental session, leak integrity was verified by sealing the chamber, flushing with test gas, and monitoring FiO_2 decline and stability; failure to maintain the expected FiO_2 profile after stopping flow was treated as evidence of leakage. Under calibrated operating conditions, the chamber FiO_2 could be reduced from approximately 21% to 5% within ~ 60 s, confirming adequate sealing and gas-switching performance (Fig. 2).

2.6 Software and Control Interface

A custom **Python 3.9 application** was developed to automate intermittent hypoxia cycling and environmental data acquisition, operating on a Linux-based Raspberry Pi OS. The system incorporates a **Tkinter-based graphical user interface (GUI)** that allows real-time visualization of chamber conditions and configuration of key protocol parameters, including cycle duration, target FiO_2 nadir, and session length.

Remote monitoring and control were enabled via **RealVNC**, permitting oversight of prolonged exposure sessions without direct interaction with the chamber and minimizing disturbance to the animals [45].

Owing to its open-source architecture, the software framework supports future expansion, including integration of additional sensors and implementation of advanced control strategies such as closed-loop feedback algorithms based on FiO_2 measurements.

Table 1.0
System components of the custom-built intermittent hypoxia chamber

Component Category	Item / Model	Brand / Manufacturer	Country of Origin	Estimated Price (SAR)
Microcontroller & Control Unit	Raspberry Pi 4 Model B (Quad-core ARM, 4 GB RAM)	Raspberry Pi Foundation	UK	350 SAR
	5-inch Raspberry Pi Touchscreen Display	Raspberry Pi Foundation	UK	200 SAR
	Breadboard and Jumper Wires	Elegoo	China	50 SAR
	Relay Module (5V, 2-Channel)	Songle / SainSmart	China	40 SAR
Gas Control System	12V DC Solenoid Valves (Model YS-12)	Ningbo King Shengda Solenoid Tech.	China	120 SAR (×3 ≈ 360 SAR)
Sensors	O_2 Sensor (Gravity I²C)	DFRobot	China	300 SAR
	Carbon monoxide safety sensor (MQ-7)	Hanwei Electronics	China	50 SAR
	Temperature & Humidity Sensor (BME280)	Bosch Sensortec	Germany	40 SAR
Hypoxia Chamber	Custom Plexiglass Chamber (100 × 40 × 60 cm, airtight, mesh floor)	Biomedical Engineering Workshop, KSU	Saudi Arabia	1,500 SAR
Software & Programming	Python 3.9 Script (Linux-based, Raspberry Pi OS)	Open source (Python Software Foundation)	International	Free
	RealVNC Viewer (License)	RealVNC Ltd.	UK	150 SAR
Estimated Total System Cost ≈ 2,990 SAR				

2.7 Hypoxia–Normoxia Cycling Protocol

Two intermittent hypoxia (IH) paradigms were employed to model moderate and severe OSA-like hypoxic exposure. **Moderate IH** consisted of cycles lasting **2–3 min**, yielding **20–30 hypoxic events per hour**, during which chamber FiO_2 was reduced to **10–12%**, followed by reoxygenation to normoxia. **Severe IH**

consisted of **1-min cycles** (up to **60 events per hour**), with FiO_2 rapidly reduced to **5–7%** and subsequently restored to normoxic levels **[46]**.

All exposures were conducted during the **light (inactive) phase** to align with the rodents' natural rest period and mimic the sleep-associated hypoxic stress of human OSA. Control animals were housed under identical experimental conditions, including chamber environment, airflow, and noise exposure, but were continuously supplied with room air (21% O_2) throughout each session.

2.8 Statistical Analysis

Descriptive data are presented as **mean ± standard deviation (SD)**. The **coefficient of variation (CV%)** was used to assess the reproducibility of chamber FiO_2 cycling across cycles and experimental days. Differences in **FiO_2 nadir values** across sessions were analyzed using **one-way analysis of variance (ANOVA)**. Statistical significance was defined as $p < 0.05$. All analyses were performed using **GraphPad Prism (version 10.0)** and **Python** (NumPy and SciPy libraries).

3. Results

3.1 Chamber Oxygen Profile

The intermittent hypoxia (IH) chamber operated reliably in accordance with the programmed protocols, with continuous monitoring confirming accurate and reproducible control of **chamber oxygen concentration (FiO_2)** throughout all exposure sessions. During the **severe intermittent hypoxia (IHS)** protocol, FiO_2 decreased from normoxic levels (21%) to the target hypoxic range of **5–7% within approximately 20–25 seconds**, depending on gas flow conditions. Reoxygenation was initiated immediately following the hypoxic phase, with FiO_2 returning to normoxia within **30–60 seconds** (Fig. 3).

Across repeated severe IH cycles, FiO_2 nadirs were consistently achieved with minimal variability. The mean FiO_2 nadir remained within the predefined hypoxic range, with a **coefficient of variation (CV) < 5%**, demonstrating stable solenoid valve actuation, effective chamber sealing, and precise gas delivery.

Similarly, during **moderate intermittent hypoxia (IHM)**, chamber FiO_2 reproducibly decreased to **10–12%** and returned to normoxia following each programmed cycle (Fig. 4). Analysis of **30 consecutive moderate IH cycles** showed tight clustering of FiO_2 nadir values within each session, confirming high **cycle-to-cycle reproducibility** and precision of the gas delivery system.

The characteristic **desaturation–reoxygenation waveform** observed in the FiO_2 traces verified accurate timing of solenoid valve switching and appropriate calibration of gas flow rates. No progressive drift in FiO_2 nadirs or recovery peaks was observed across sessions, indicating consistent chamber performance over time.

3.2 Environmental Conditions and Stability

Continuous environmental monitoring facilitated maintenance of stable chamber conditions during prolonged exposure sessions. Ambient temperature remained tightly controlled, with an average value of **22.5 ± 0.3°C**, while temperature within the chamber was maintained at approximately **22.4°C** throughout the experiments. Relative humidity remained stable during the 8-h exposure periods, typically ranging between **55% and 80%**. A transient increase in humidity was observed following animal placement, which normalized within approximately **1 hour**, consistent with animal respiration and chamber ventilation dynamics.

Carbon monoxide sensing was employed **solely as a qualitative safety indicator** to detect abnormal gas accumulation associated with ventilation failure. Under normal operating conditions with continuous outflow, no abnormal accumulation signal was detected, indicating effective chamber flushing. In contrast, preliminary validation tests conducted **without active outflow** resulted in rapid gas accumulation signals within approximately **30 minutes**, underscoring the importance of continuous ventilation for maintaining chamber air quality.

Noise levels generated by solenoid valve actuation during gas switching were approximately **50–60 dB**. Animals habituated rapidly to these sounds, with no overt behavioral signs of sustained stress observed after the initial exposure period.

Collectively, these findings demonstrate that the chamber maintained a **stable and controlled microenvironment** during prolonged intermittent hypoxia exposures, effectively isolating **oxygen concentration (FiO_2)** as the primary experimental variable (Fig. 5).

3.3 System Performance and Reliability

The system performed consistently across multiple **8-hour daily runs**. Raspberry Pi–based control software exhibited valve-switching accuracy with **< 1-second cumulative drift** over several hours of continuous operation. Relay modules and solenoid valves completed **> 10,000 actuation cycles** without mechanical or electrical failure, indicating robust long-term durability.

Continuous data logging yielded approximately **2,880 FiO_2 measurements per 8-hour session** (1-second sampling interval), providing high-resolution oxygen concentration traces for system validation. Over **seven consecutive days of exposure**, FiO_2 nadirs exhibited negligible drift, with a **standard deviation ≤ 0.5% O_2**.

One-way ANOVA revealed **no significant between-day differences in FiO_2 nadir values** ($p > 0.5$), confirming reproducibility of hypoxia–normoxia cycling across repeated sessions (Table 2). Minor FiO_2 overshoot observed in early prototype testing was resolved by optimizing reoxygenation flow rates and incorporating a passive bleed valve. These refinements eliminated transient hyperoxic excursions (> 21% O_2) and improved pressure stability during gas infusion.

Overall, the system demonstrated **durable, hands-off, and highly reproducible performance**, supporting its suitability for long-term intermittent hypoxia experimental protocols.

Table 2
System performance metrics are summarized in demonstrating stable FiO_2 control, hardware durability, and reproducible operation across multi-day exposure sessions.

Performance Metric	Measurement / Outcome	Validation Context
Exposure duration per session	8 hours	Continuous unattended operation
Control software timing drift	< 1 s over several hours	Raspberry Pi–based Python control
Solenoid valve actuation cycles	> 10,000 cycles	No mechanical or electrical failure observed
Relay module performance	Stable	No switching errors or overheating
FiO_2 sampling rate	1 sample per second	Continuous chamber-level monitoring
FiO_2 data points per session	~ 2,880 readings	Per 8-hour exposure
Severe IH FiO_2 nadir range	5–7% O_2	Achieved within 20–25 s
Moderate IH FiO_2 nadir range	10–12% O_2	Stable across cycles
FiO_2 nadir variability (day-to-day)	SD ≤ 0.5% O_2	Over 7 consecutive days
FiO_2 reproducibility across days	No significant difference (ANOVA, $p > 0.5$)	Confirms session-to-session stability
FiO_2 overshoot above normoxia	None after optimization	Bleed valve + flow tuning
Pressure stability during infusion	Stable	No detectable pressure spikes
Data logging reliability	100%	No data loss observed
Long-term operational reliability	High	Suitable for multi-day IH protocols

4. Discussion

The present study demonstrates that a low-cost, open-source intermittent hypoxia (IH) chamber can achieve precise, stable, and reproducible control of chamber oxygen concentration (FiO_2) during prolonged and repeated exposure sessions. The system reliably generated moderate and severe IH profiles with rapid desaturation and reoxygenation kinetics, minimal variability across cycles, and

negligible drift across multiple days. These findings confirm that robust engineering performance can be achieved using widely available components and open-source software, providing a practical alternative to high-cost commercial platforms.

4.1 FiO_2 Control and Reproducibility in Relation to Existing IH Systems

A critical requirement for IH research is the ability to achieve rapid and repeatable oxygen transitions that approximate the hypoxia–reoxygenation dynamics used in rodent models of obstructive sleep apnea (OSA) [47]. In the present system, severe IH protocols consistently reduced FiO_2 from 21% to 5–7% within approximately 20–25 s, with recovery to normoxia within 30–60 s. Moderate IH cycles reproducibly achieved nadirs of 10–12% FiO_2 with cycle-to-cycle variability below 5%.

These transition kinetics and reproducibility metrics are comparable to those reported in validated rodent IH studies using both commercial hypoxia systems and custom-built chambers [18]. Commercial platforms such as the OxyCycler provide high precision but rely on proprietary hardware and closed software ecosystems, limiting accessibility and customization [48]. In contrast, the present system achieved comparable FiO_2 control while remaining fully open-source and substantially less expensive, facilitating broader adoption and protocol transparency.

4.2 Comparison with Published Open-Source and Custom IH Chambers

Several open-source or low-cost IH chamber designs have been described in recent years, each addressing specific limitations of commercial systems. Gaspar et al. developed a microcontroller-based hypoxia chamber emphasizing affordability and automation but lacking comprehensive environmental monitoring and scalability [50]. Hillman et al. introduced an automated system with PID-based oxygen control, achieving high precision but restricting experiments to single-animal exposures [49]. Roshan et al. reported a compact Arduino-based design optimized for simplicity, though without integrated environmental sensing or multi-animal capacity [50].

Compared with these systems, the present chamber advances the field in several respects. It supports **multi-animal exposure (up to 10 rats)** while maintaining stable FiO_2 kinetics, integrates **continuous oxygen, temperature, and humidity monitoring**, and provides **real-time graphical control** through a Raspberry Pi interface. These features enhance experimental throughput, reproducibility, and usability relative to previously published open-source IH chambers [24].

4.3 Environmental Stability and Control of Experimental Confounders

Beyond oxygen control, maintaining a stable chamber microenvironment is essential for isolating hypoxia as the primary experimental variable. Continuous monitoring demonstrated tight regulation of chamber temperature (~22.4–22.5 °C) and stable relative humidity (55–80%) during prolonged 8-h exposures. Transient humidity increases following animal placement resolved within approximately one hour, consistent with expected respiratory moisture contributions and effective ventilation.

Continuous chamber flushing prevented abnormal gas accumulation, as indicated by qualitative safety monitoring, and solenoid-related noise (~50–60 dB) was rapidly tolerated by animals. These findings are consistent with previous reports showing that uncontrolled temperature, humidity, or ventilation can confound physiological outcomes in long-duration IH studies [51].

4.4 System Durability and Long-Term Performance

The system demonstrated strong durability during extended use, completing more than 10,000 solenoid valve cycles without mechanical or electrical failure. Control software-maintained valve-switching accuracy with less than one second of cumulative timing drift over several hours. High-resolution FiO_2 logging (1-s sampling) enabled detailed validation of oxygen profiles across sessions.

Across seven consecutive days, FiO_2 nadirs remained stable with no significant between-day differences, confirming long-term reproducibility. Minor hyperoxic overshoot observed in early prototypes was eliminated through flow optimization and incorporation of a passive bleed valve, highlighting the importance of iterative engineering validation in IH system development [16].

4.5 Scope and Limitations

The present work constitutes an **engineering validation** of chamber performance rather than a physiological validation of animal responses. While precise chamber-level FiO_2 control is a prerequisite for IH research, biological outcomes depend on animal-specific factors such as ventilation, perfusion, activity, and metabolic rate [52]. Accordingly, downstream physiological and molecular measures remain essential when applying this platform to specific disease models.

4.6 Conclusions

In summary, the developed low-cost, open-source IH chamber provides precise, stable, and reproducible control of chamber oxygen concentration with integrated environmental monitoring and robust long-term performance. Compared with both commercial and previously published open-source systems, it offers a favorable balance between affordability, scalability, transparency, and engineering rigor. This platform has the potential to expand access to standardized intermittent hypoxia paradigms and support reproducible preclinical research across diverse laboratory settings.

Abbreviations

Abbreviation	Definition
OSA	Obstructive Sleep Apnea
IH	Intermittent Hypoxia
FiO_2	Fraction of Inspired Oxygen
SpO_2	Peripheral Oxygen Saturation
CPU	Central Processing Unit
GUI	Graphical User Interface
GPIO	General-Purpose Input/Output
IHM	Moderate IH Protocol
IHS	Severe IH Protocol
CV	Coefficient of Variation
SD	Standard Deviation
RH	Relative Humidity

Declarations

Author Contribution

Author Contributions StatementS.A.A. conceived and designed the study, developed the intermittent hypoxia chamber, performed system validation, conducted data acquisition, and drafted the original manuscript. A.A.A.-M. provided scientific supervision, conceptual guidance, and critical revision of the manuscript. S.A.A. contributed to the physiological interpretation of the data and manuscript review. assisted with experimental planning and data interpretation. M.A.M. contributed to the engineering design, fabrication, and technical optimization of the chamber. contributed to methodological refinement and critical manuscript editing.All authors reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Ethical Approval and Animal Welfare

All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of King Saud University, in accordance with the Guide for the Care and Use of Laboratory Animals (8th Edition, National Academies Press, 2011). To minimize stress, rats were acclimatized to the chamber before the intermittent hypoxia exposure. Light–dark cycle, temperature, and humidity were kept constant, and the well-being of the animals was observed throughout all the sessions.

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Data Availability Statement

The open-source hardware designs and control software developed in this study are available from the corresponding author upon reasonable request. Experimental datasets generated during the study are not publicly available due to institutional and ethical constraints but can be accessed from the corresponding author upon reasonable request.

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Figures

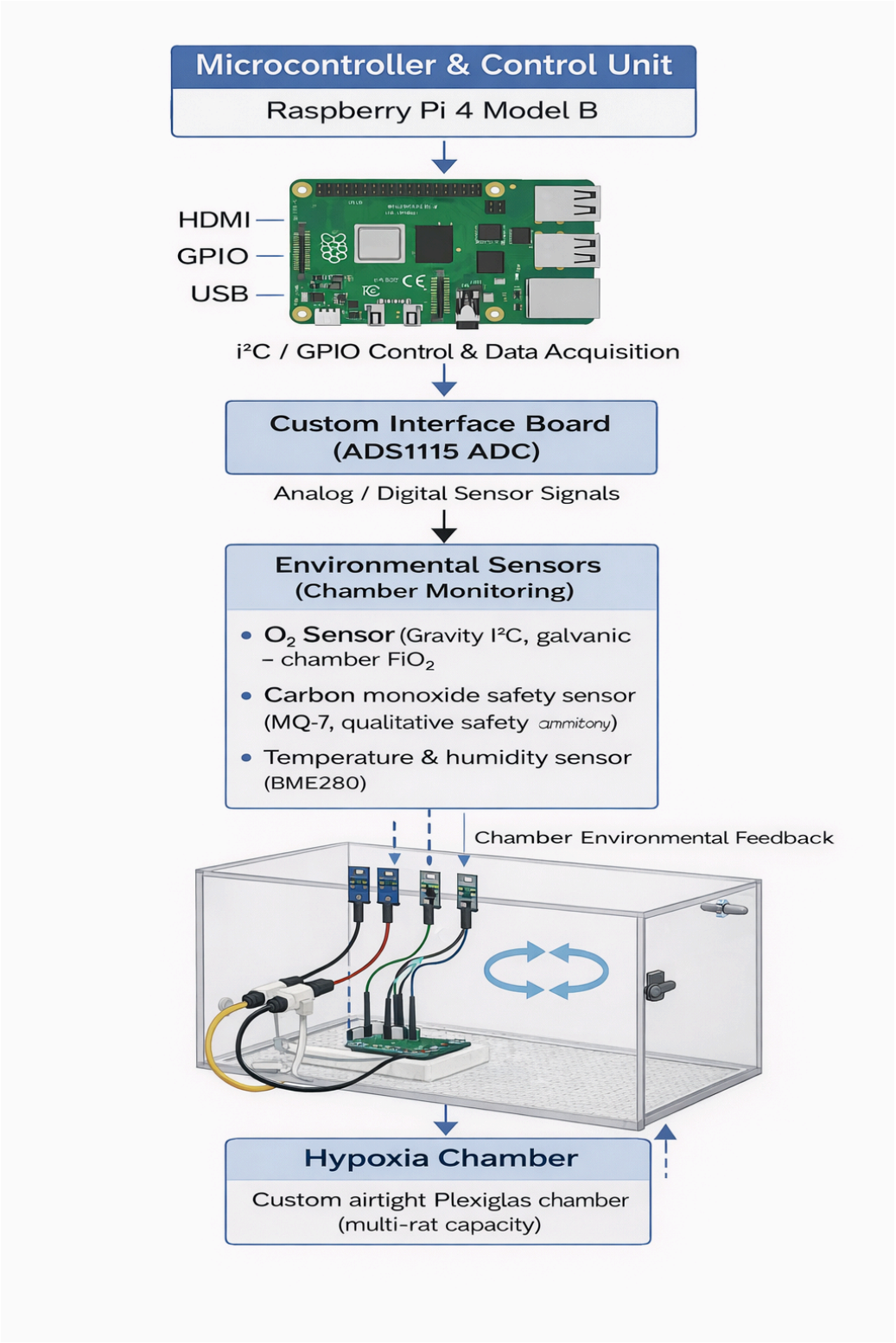
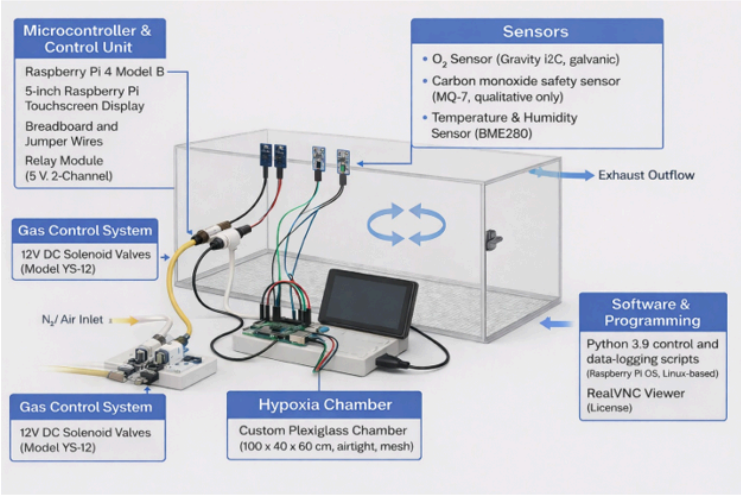


Figure 1

Electronic architecture and sensor integration of the open-source intermittent hypoxia(IH)chamber. The system is controlled by a Raspberry Pi 4 Model B interfaced with a custom signal-conditioning board (ADS1115 ADC) for sensor acquisition. Environmental monitoring includes a galvanic oxygen sensor for chamber FiO_2 measurement, a carbon monoxide safety sensor (MQ-7) used exclusively for qualitative safety monitoring, and a temperature and humidity sensor (BME280). All sensors measure chamber

environmental conditions only. Sensor data are processed and logged via Python-based software, enabling real-time monitoring and automated hypoxia–normoxia cycling.



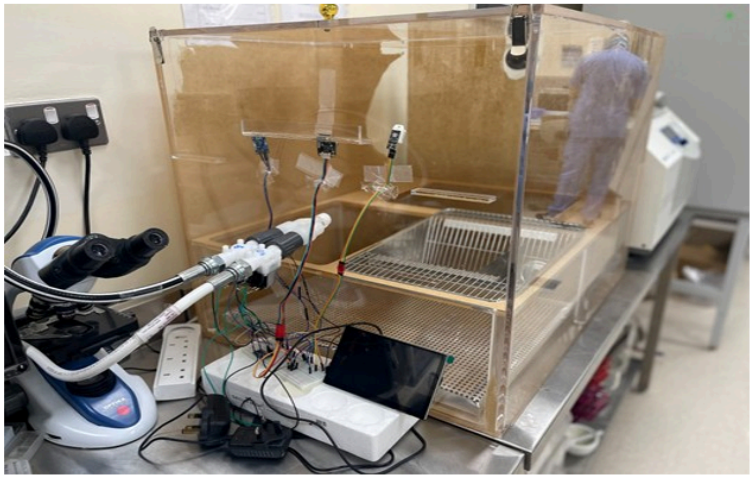




Figure 2

Schematic overview of the custom-built open-source intermittent hypoxia (IH) chamber.

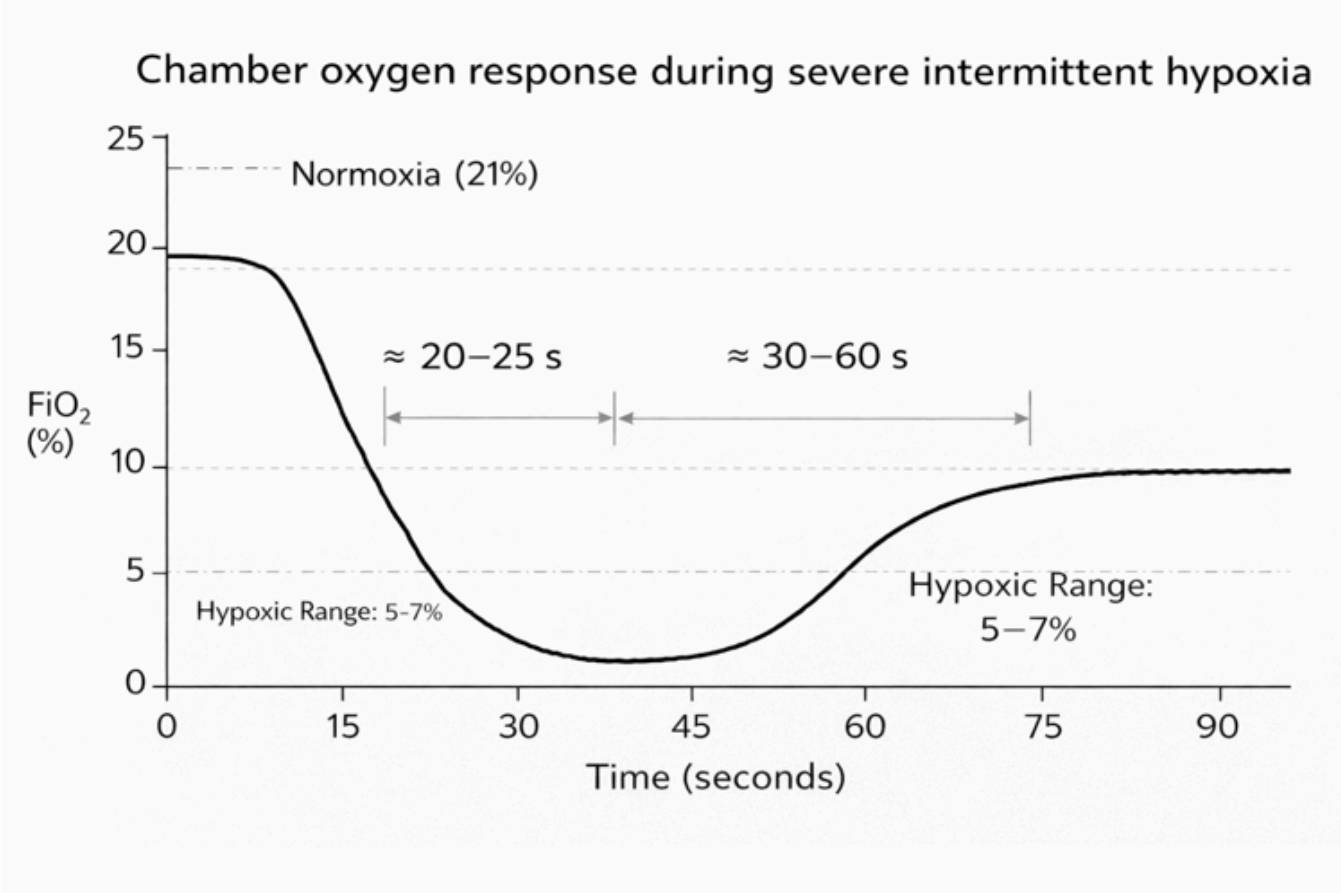


Figure 3

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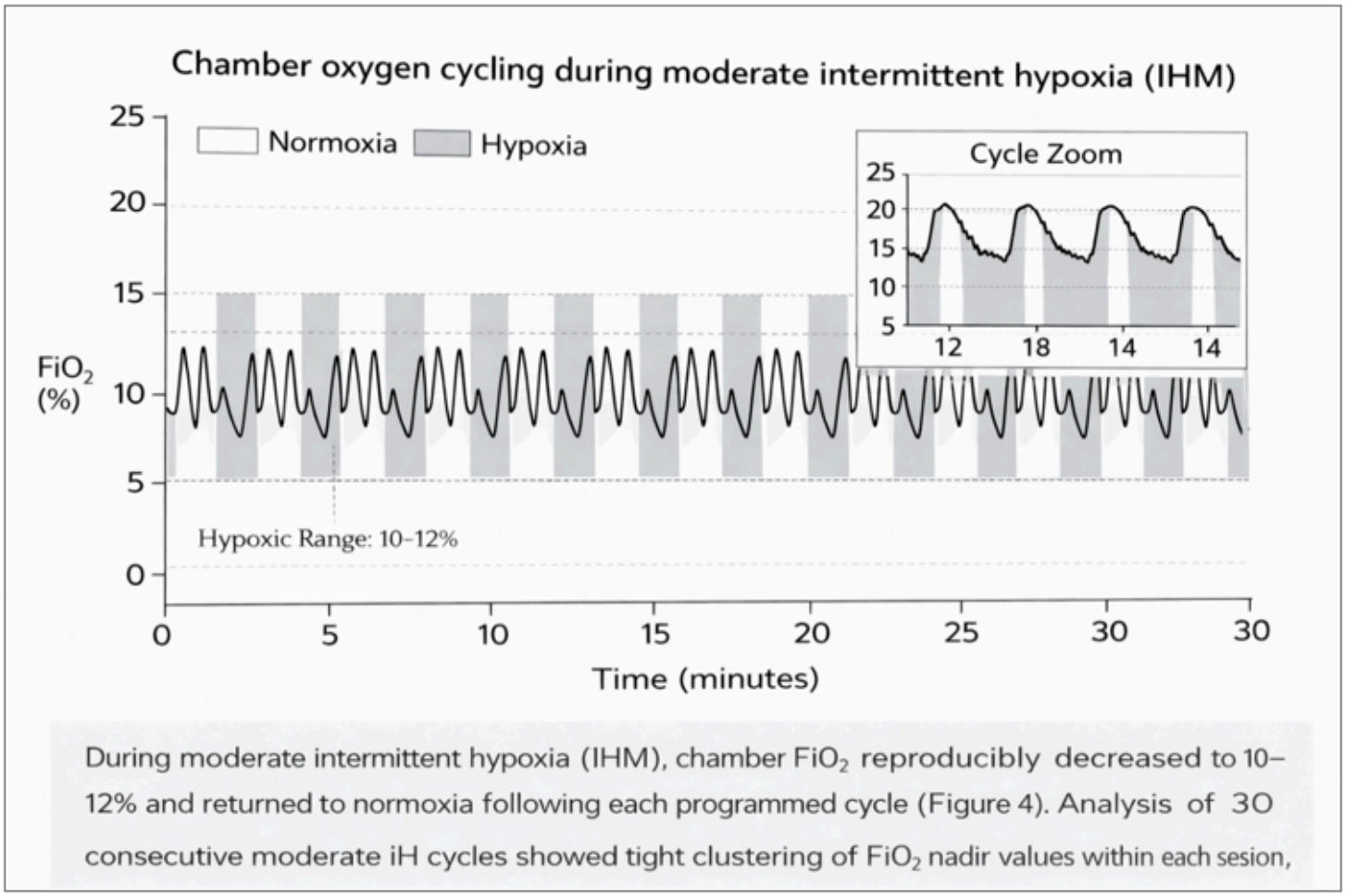
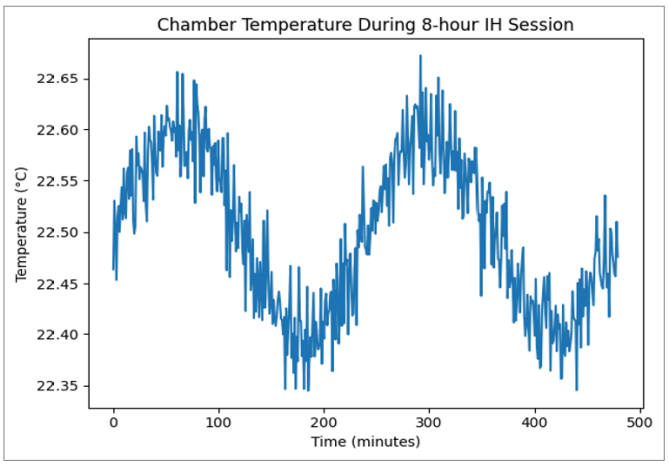
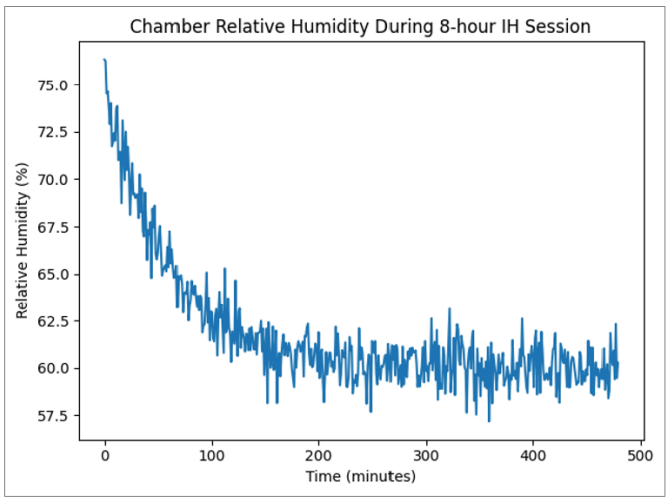


Figure 4

Legend not included with this version.



A



B

Figure 5

A Chamber Temperature vs Time: Stable temperature maintained around **22.5 ± 0.3 °C**, Minor physiological fluctuations only, No drift across the **8-hour IH session**

B: Chamber Relative Humidity vs Time: Initial transient rise in humidity after animal placement**,** Gradual stabilization within ~1 hour**,** Maintained within **~55–80% RH** for the remainder of exposure