Article title

Optimized open-source setting for subjecting rodents to chronic normobaric hypoxia in facilities with minimal nitrogen supply.

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Abstract

Very prevalent respiratory and cardiovascular diseases result in chronic hypoxia, promoting metabolic, kidney, heart, and other malignant diseases. Hypoxia research employs animal models based on chronically breathing hypoxic air (O_2<21%), usually by injecting N_2 into the animal's chamber. However, continuous high-flow N_2 supply is available only in limited facilities, reducing the capability of widely conducting hypoxia research. Here, we describe an optimized setting for subjecting rodents to chronic normobaric hypoxia by requiring minimal N_2 supply. The setting is based on providing the O_2 consumed by the animals and eliminating the exhaled CO_2 and water vapor. O_2, CO_2, temperature, and humidity in the hypoxic chamber are controlled by an Arduino-based unit activating a pump that introduces room air to restore the metabolized O_2. Another pump continuously recirculates the chamber air through a Peltier-based drier and CO_2-absorbing soda lime. To correct any deviation in the actual value of hypoxia within the chamber, the control unit allows the injection of N_2 into the chamber from a gas source. The setting performance was successfully tested *in vivo* when subjecting mice to 11%-O_2 chronic hypoxia. This device, requiring a low N_2 supply, may facilitate *in vivo* experimental research of hypoxiarelated diseases.

Keywords

Oxygen sensor, animal research, gas concentration control, CO_2 monitoring, hypoxia model.

Specifications table

Hardware name	Device for subjecting rodents to chronic hypoxia with minimal nitrogen supply
Subject area	Experimental biology and biomedicine
Hardware type	Device for biomedical animal research
Closest commercial analog	No commercial analog is available
Open source license	GPL v3
Cost of hardware	The total cost of the material for the device building is ≈ 500 US$.
Source file repository	https://data.mendeley.com/datasets/t7dk933sjm/1

1. Hardware in context

Respiratory and cardiovascular diseases are nowadays among the most prevalent ones (1,2), and their high morbidity and mortality are expected to increase further because of the worldwide current epidemics of obesity (3,4) and the rise in life expectancy (5,6). Owing to either poor air oxygenation in the lungs or inadequate blood circulation and distribution, these diseases usually result in chronic hypoxia, a state of poor oxygenation of the cells in tissues and organs. Hypoxia severely affects normal cell function, resulting in negative consequences in multiple organs, such as myocardial ischemia, metabolic diseases, chronic heart and kidney diseases, reproductive diseases, and cancer (7). Hence, hypoxia plays a relevant pathophysiological role in human health and is a subject of intense investigation (8).

Research on the mechanisms involved in the multiorgan consequences of normobaric hypoxia requires animal models based on chronically breathing air with O_2 concentration below the usual 21% of atmospheric air. The most common experimental setting for achieving hypoxia in animal models is to place them into a chamber where the O_2 concentration in the ambient air is reduced by injecting a flow of N_2 (9,10). Indeed, regulating the flow of room air and of N_2 entering the chamber is the simplest way to control the concentration of O_2 the animals breathe. However, a relatively high flow of N_2 is required since, to prevent hypercapnia, the injected gas must sufficiently wash out the CO_2 exhaled by the animals.

In most of the cases, the source of N_2 required for the most conventional setting to continously subject animals to hypoxia cannot be based on conventional bottles of compressed gas because of the high consumption required. A possible option would be an N_2 generator (based on N_2 extraction from room air by a pressure swing adsorption concentrator). However, these devices are expensive, limiting their use for this application. The most common alternative is a centralized N_2 gas pipeline system installed in the building to provide gas to different points of use. However, this infrastructure, which is commonly available in hospitals and cell biology laboratories, is usually unavailable in most animal laboratory facilities. Such requirements for a continuous N_2 source limit the widespread extension of hypoxia-related *in vivo* research.

To facilitate the research employing hypoxic animal models in facilities not having access to a continuous high-flow N_2 source, we aimed to design, build, and test an open-source, low-cost device requiring minimal N_2 provision.

1. Hardware description

2.1. Principle of functioning

Contrary to most conventional settings for producing hypoxic air by continuous N_2 injection, the device described here aims to minimize the supply of N_2 when subjecting mice to controlled chronic hypoxia. The setting is based on ensuring the balance of the gases involved in mice metabolism, thus providing the O_2 consumed and eliminating the exhaled CO_2 and water vapor. A schematic description of the setting is presented in Figure 1: The O_2 and CO_2 concentrations, the relative humidity (RH), and temperature (T) inside the hypoxic chamber are continously measured by sensors, and processed by the control and display unit, based on an Arduino microcontroller. A high-resistance air leak communicates the hypoxic chamber with the atmospheric air. The air is continously recirculated within the chamber by the use of a domestic aquarium pump. A soda lime filter is used to absorb the generated CO_2 and a drier/cooler (based on a Peltier unit providing cooled water that circulates through an air radiator) is used to regulate the temperature and to condense water vapor. For this purpose, the drier/cooler section is enclosed into a thermally isolating box made with polystyrene walls including with an outlet to extract the condensed water.

A second domestic aquarium pump is used to introduce room air into the hypoxic chamber to supply the O_2 consumed by the mice. Ideally, the setting would not require any additional N_2 injection to keep a target level of hypoxia since the consumption of O_2 and the production of CO_2 and water vapor are balanced by the components of the setting (Figure 1). Howeverthe control unit allows the injection of N_2 into the chamber from a gas bottle through an electrically controlled valve.

The specific device we implemented (Figure 2) was designed to expose up to 30 adult mice to chronic normobaric hypoxia. The hypoxic chamber dimensions were 50 x 61 x 84 cm, allowing it to easily accommodate 6 conventional mice cages (17 x 20 x 39 cm) for 5 animals each. The design of the setting was based on the analysis of the physiological variables corresponding to the demanding conditions of 30 mice with the highest possible adult weight of 40 g each, as explained in the following subsections. The dimensions and specifications of the different components can be modified as required if changing the number of mice or the animal species (e.g., rats or guinea pigs).

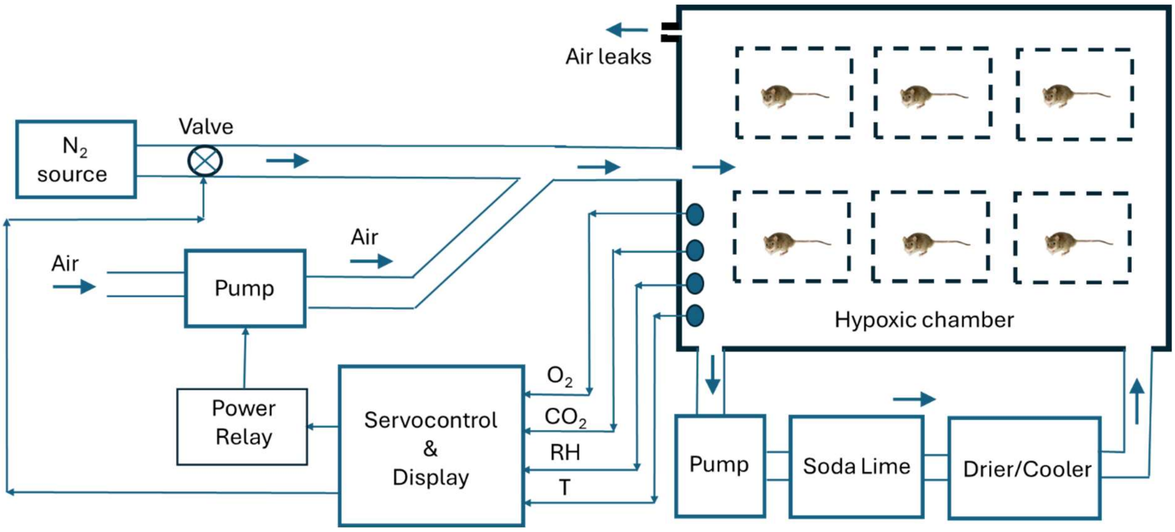


Figure 1. Diagram of the hypoxia setting for mice. RH and T are relative humidity and temperature, respectively. O_2 and CO_2 indicate gas concentrations. See text for explanation.

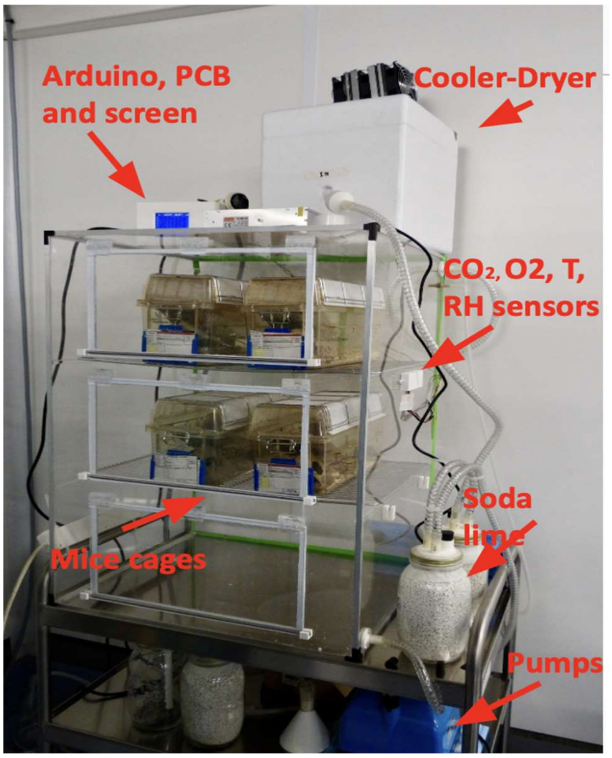


Figure 2. Picture of the implemented hypoxic setting with capacity for 30 mice.

2.2. Oxygen balance

The typical rate of O_2 consumption in a mouse is 0.06 ml·(min·g)$^{-1}$)(11). Therefore, the total oxygen flow consumed (V'_{O2}) by the mice is ≈ 72 ml·min^{-1}. The value of oxygen fraction (F_iO_2) in the hypoxic chamber that the user sets on the front panel of the control unit is achieved by mixing the flow of room air (V'_{air}) and the N_2 flow (V'_{N2}) entering from the N_2 source. The O_2 flow entering the chamber is the amount introduced by the air pump (21%·V'_{air}). The total flow of O_2 leaving the chamber is the addition of metabolic consumption (V'_{O2}) and the O_2 contained in the total gas leaving the chamber (V'_{N2}+V'_{air}) which is at F_iO_2. Thus, $0.21 \cdot V'_{air} = V'_{O2} + F_iO_2 \cdot (V'_{air} + V'_{N2})$, and hence $V'_{air} = (V'_{O2} + V'_{N2} \cdot F_iO_2) / (0.21 - F_iO_2)$. After an initial injection of N_2 to achieve the desired level of hypoxia, e.g. a target $F_iO_2 = 0.11$, minimization of further N_2 consumption ($V'_{N2} \approx 0$) during steady-state hypoxia would require V'_{air} = 720 ml·min^{-1} for V'_{O2} = 72 ml·min^{-1}. This F_iO_2 value is, in practice, ensured by using the O_2 sensor signal to control the power of the pump injecting the airflow V'_{air} into the chamber.

2.3. CO_2 balance

A critical condition to be achieved inside the hypoxic chamber is normocapnia since the setting would tend to induce hypercapnia by the potential accumulation of the CO_2 produced by mice metabolism. Assuming that the ratio between CO_2 production and O_2 consumption, commonly known as respiratory exchange rate (*RER*), is equal to 1 in mice (11), the total flow of CO_2 metabolically produced (V'_{CO2}) is ≈ 72 ml·min^{-1}. In case the CO_2 fraction in the chamber (F_iCO_2) was simply the result of the washout induced by the total circulating air ($V'_{air} + V'_{N2}$), i.e. no CO_2 absorption by soda lime, the CO_2 entering the chamber by mice metabolism would be balanced by the CO_2 leaving the chamber. Under a steady state with minimal N_2 supply ($V'_{N2} \approx 0$, V'_{air} = 720 ml·min^{-1}), CO_2 washout would be negligible. Accordingly, the only effective way to reduce CO_2 accumulation in the chamber is by continuously pumping a flow (V'_{sl}) of the chamber air through a soda lime canister acting as a CO_2 absorber. Then, the CO_2 metabolically produced (V'_{CO2} = 72 ml·min^{-1}) is balanced by the CO_2 absorbed by the soda lime ($V'_{sl} \cdot F_iCO_2$) and the negligible amount of CO_2 leaving the chamber ($V'_{air} \cdot F_iCO_2$). Hence, for a pump flow V'_{sl}= 60 l·min^{-1}, the increase in F_iCO_2 would be of only ≈ 72 ml·min^{-1}/60000 ml·min^{-1} = $12 \cdot 10^{-4}$ or 1200 ppm (0.12%) above the typical F_iCO_2 of room air, hence a safe value (12). Since 1 kg of soda lime can absorb up to 260 l of CO_2 (13), the soda lime required to drain the 72 ml·min^{-1} of CO_2 production is 520 g·day^{-1}.

2.4. Water vapor balance

Typical ventilation in mice is 1.46 ml·g^{-1} (14), thus the total respiratory minute volume (V'_{min}) is ≈ 1752 ml·min^{-1}. Water vapor is released by mice metabolism, mainly through breathing, thus tending to increase the relative humidity (*RH*) in the chamber air. Expiratory air (at 37 °C and water vapor saturated) and inspiratory air (at chamber temperature and RH_b) contain different amounts of water vapor. Taking into account that the partial pressure of water vapor ($P_{H2O,sat}$) at 37 °C is 47 mmHg, for V'_{min} = 1752 ml·min^{-1}, the water vapor content in the exhaled air is 1752 ml·min^{-1} · (47 / 760) = 108.3 ml·min^{-1}. As $P_{H2O,sat}$(25°C) = 23.8 mmHg, the water vapor in the inhaled air content is 1752 ml·min^{-1}·(23.8 / 760) · RH_b = 54.9 · RH_b ml·min^{-1}. Hence, the net water vapor produced by breathing is the difference content in expired and inspired air (108.3 − 54.9 · RH_b) ml·min^{-1}. Thus, to keep RH_b at a reasonable value of 60%, a common value in lab animal facilities, the water vapor flow to be eliminated is 75.4 ml/min. Adding the water vapor released by mice transepithelial evaporation (which amounts to ≈ 50% of water vapor loss by breathing (15), ≈ 113 ml/min of water vapor should be eliminated by the air drier to avoid excessive humidification of the hypoxic chamber air. This amount of water vapor is eliminated by condensation in a the cool drier, specifically by cooling the airflow (V'_{sl} =60000 l/min) that is already circulated through soda lime to maintain normocapnia. Indeed, if a flow (V'_{sl}) of chamber air (RH_b = 60%, 25 °C, thus containing 1128 ml/min of water vapor) is circulated through a refrigerated element that cools the air to a temperature (T_c), the maximum content of water vapor in the refrigerated air will be reduced and thus the exceeding amount will condensate on the inner walls of the cooler. The maximum flow of water vapor contained by saturated cooled airflow V'_{sl} at T_c is 60000 ml/min · $P_{H2O,sat}(T_c)$ / 760 = 79 ml/min · $P_{H2O,sat}(T_c)$. Hence, the liquid water condensed by cooling V'_{sl} from 25 °C to T_c is 1128 – 79 · $P_{H2O,sat}(T_c)$. Accordingly, to eliminate the ≈ 113 ml/min of metabolically-produced water vapor, it is required that $P_{H2O,sat}(T_c)$ = 12.8 mmHg, corresponding to Tc ≈ 15 °C. Hence, a relatively mild ≈ 10 °C refrigeration from 25 °C would be enough.

2.5. Head transfer balance

The metabolic heat dissipation by 25-g mice at common ambient temperature (20-25 °C) is 0.5 Kcal/h (11), and thus the heat dissipated by the 30 mice (40 g each) would be (Q'_{met}) is ≈ 30 W. The net heat balance in the hypoxic chamber is determined by the positive 30 W metabolically dissipated by the mice, the heat dissipated by the soda lime in the absorption process of CO_2 (13.7 kcal/mol$_{CO2}$) (13), which in the setting amounts 2.8 W (for CO_2 density 1.8 g·l^{-1} at 20 °C), and the negative heat transfer required for heating the previously cooled airflow V'_{sl} entering the chamber from 15 °C to 25 °C, which amounts –12.0 W (as computed for air density 1.2 g·l^{-1} and specific heat capacity 1 J·g^{-1}·K^{-1}). Hence, the net heat balance is ≈ 21 W. Taking into account that the chamber (50 x 61 x 84 cm) has a surface (A) of 2.47 m^2, that the chamber walls are made of 4-mm width (d) transparent polymethyl methacrylate (thermal conductivity: K = 0.18 W·m^{-1}·K^{-1}), and that the basic equation for heat transfer is $Q' = K \cdot A \cdot \Delta T \cdot d^{-1}$, it results that passive dissipation of Q' = 21 W through the chamber walls would be achieved for a $\Delta T \leq 0.2$ °C. Hence, heat transfer balance is achieved for a hypoxic chamber temperature that minimally differs from room temperature.

2. *Design files summary*

All the design and software files necessary to build the LAMP device presented in this work are distributed under the GPL v3 license and they can be found in the supplementary materials of this manuscript at the following public repositories (DOI: 10.17632/t7dk933sjm.1)

https://data.mendeley.com/datasets/t7dk933sjm/1

Table 1. Files summary

Design file name	File type	Open source license	Location of the file
Enclosures and lids	STL	GPL v3	STL files folder
Code	ino file	GPL v3	Arduino Code folder
PCB Layout	pdf and jpg file	GPL v3	Electronics folder

3. Bill of materials summary

Component	Quantity	Cost per unity €	Total Cost currency €	Source of materials
Sensor O_2	*1*	62,30	62,30	https://es.farnell.com/dfrobot/sen0322/i2c-oxygen-sensor-module-arduino/dp/3879708?gad_source=1&CMP=KNC-GES-GEN-SHOPPING-Pmax-Catch-all-05-Dec-23&gross_price=true
Sensor CO2, temperature and relative humidity	1	61,14	61,14	https://es.farnell.com/seeed-studio/101020952/m-dulo-sensor-arduino-raspberry/dp/4007751?st=modulo%20sensor%20%20co2
Voltage regulator 5V and 9V	2	0,28	0,56	https://www.amazon.com/valores-Paquete-regulador-positivo-corriente/dp/B07T5ZHY63/ref=sr_1_1_sspa?__mk_es_US=%C3%85M%C3%85%C5%BD%C3%95%C3%91&crid=1651XJCJIY90X&dib=eyJ2IjoiMSJ9.z7VZ01yDMzS7FNoFVZYjfIIDIqJf7MWuKacqC0FVcexkbaaq2K3koWWNRLSjCUnNocgOZhSxSL_K2NLBBZrMZcpmaEX61JZhaHNEK6GLg-pEYKA2nXRwSKnqndWUS3hKDuTBenbCbF9ouzxqIJhS3vUE_hhOFrJp8TIc9ngNTITIqc6fLt1BAs_ijswPUxupFI1jSGK1IUobc-yQ_mRvKIzIJFYo4byvBh0iwoiQtwk.e5isTzn0mwmTiPh46biH3ZfvGoMPDzyBZDeiYAUNsKQ&dib_tag=se&keywords=voltage+regulator+7809&qid=1749637707&sprefix=voltage+regulator+780%2Caps%2C158&sr=8-1-spons&sp_csd=d2lkZ2V0TmFtZT1zcF9hdGY&psc=1
Diode	1	0,04	0,04	https://www.amazon.com/conmutaci%C3%B3n-miliamp-voltios-silicio-electr%C3%B3nicos/dp/B07Q4F3Y5W/ref=sr_1_1_sspa?crid=19S08TO69SSPJ&dib=eyJ2IjoiMSJ9.G4ZHuurkn3IVHsyuKzuoxxIKGXN42qvZAc9mcrW5r69d231gJdkC9262W6Y9Ge7VLNqRy653RUZFWEvndlAY5xd59nQGcFeStKJK1_vYzKzwS6160cq9R0J4r7gITSLqXn6BMuL67mi8kVQTMqTT2NHGUpL0ptQOIsJUrpLD2EnGLzXHuZAQ9FXGqVdy_-a-TAHyH_NyhFEjnBrFpiu8voqXx_8EnP7By8SCqog0cLuR0ymxSKV6cAbdcwyaRp9ebK8oHe41a7807tuTpKDn0sVd7oFUAog0Dy4GwfcQmjw.AWgWGJvW0Wjxyzk9oo0Ga_ChksybQGJP_y22Lfu2cvI&dib_tag=se&keywords=in4148&qid=1749637823&sprefix=%2Caps%2C115&sr=8-1-spons&sp_csd=d2lkZ2V0TmFtZT1zcF9hdGY&psc=1
Transistor	1	0,28	0,28	https://www.amazon.com/valores-transistor-potencia-epitaxial-Darlington/dp/B08BFYVK6C/ref=sr_1_2_sspa?__mk_es_US=%C3%85M%C3%85%C5%BD%C3%95%C3%91&crid=F7L9BPIUJJ1J&dib=eyJ2IjoiMSJ9.h3c-xIy3MyTNjNTX3uCVtQcwfWZK_sKfZ5uAxLmZxsqRtPa0gU6bxTYAyV5DnWuyAXByYs1n1nX0i6Q_I5MTe88pNAxBZxrPfEN8voxaQCtiZdJVnQwGgVRG-ODWzeXTkUsDoi6EbqcQMcgAnbQ2VqfPOWZgFRMchaxT0K9n-Gp6LGWk1V-iedqbZIP-o_bcyz1OLHvcxVl1vjiv74yYgciAfzE5yGDDIyRHC4qiHCw.mcSg2LgzvVTPouRlbtI4MDXSFdDEva1NHrZGLCBTpZU&dib_tag=se&keywords=tip122&qid=1749637875&sprefix=tip12%2Caps%2C198&sr=8-2-spons&sp_csd=d2lkZ2V0TmFtZT1zcF9hdGY&psc=1
Solid state Relay	1	2,20	2,20	https://www.amazon.com/-/es/HiLetgo-m%C3%B3dulo-estado-Control-fusible/dp/B00WSN9CJC/ref=sr_1_6?__mk_es_US=%C3%85M%C3%85%C5%BD%C3%95%C3%91&crid=1DCIOST74VCPI&dib=eyJ2IjoiMSJ9.q91PYm6OKAKVxQl4ebBf0AcV_wFfLUw6yFpImY74y9NNuAwuMA_hpIqRP2tM66p4flq29UABM9fA-Vt8GxLx1_6p2ie44_AJ84mtp5V95vCcb2_E7tkDR2bruMwsij8DGnkhkGYR3yGb3K202Yot5Uw3FOzQ5Em4ew4ZXLH8U5mn9tf4yMoJdQZ_

				yI1NPchNJZzmKsWA_Omo89YqgdAJFDpTr6nQ7RvzFNO0pdqxyZg.B-yP_CUBkbA0UKhlF3XE1GJNXnzZo4iaBUsoYxd5gl0&dib_tag=se&keywords=rele+solid+state+5V&qid=1749637984&sprefix=rele+solid+state+5v%2Caps%2C131&sr=8-6
Conectores	6	0,11	0,66	https://www.amazon.com/s?k=conectores+arduino&__mk_es_US=%C3%85M%C3%85%C5%BD%C3%95%C3%91&crid=ZOJJGKBHPROE&sprefix=conectores+arduino%2Caps%2C133&ref=nb_sb_noss
Capacitors	4	0,04	0,16	https://www.amazon.com/ALLECIN-surtido-condensadores-electrol%C3%ADticos-aluminio/dp/B0C1VBXCQM/ref=sr_1_1_sspa?__mk_es_US=%C3%85M%C3%85%C5%BD%C3%95%C3%91&crid=O5JYUB0HNKY2&dib=eyJ2IjoiMSJ9.ekYH7jx2EeGYfnpDMkTUbA5_8nAZM5NXRF7ISTCDYRdiGRRate4MlrQd9ldvZs-A_pzD-zjApyYWkhjoUOqNALpVcJsc6DD3JHlReRoBcxMOy5HnZgoPPDGKGQ7eKIroLYErlCkXUuGwYEHo_DbuP4dZ3WHupx5R5NgErb4ax20-0bVA56GPbf6AHFW0x2BVjzMCRqJcM72pPARxXIipVzthtF2eEvVnVx2oaxKJv7E.rax_WABdj5Kg3iZg0-DCwqXoqhX58FBhrXxgFDcFrrk&dib_tag=se&keywords=condensadores&qid=1749638632&sprefix=condensadore%2Caps%2C169&sr=8-1-spons&sp_csd=d2lkZ2V0TmFtZT1zcF9hdGY&psc=1
Arduino Mega	1	23,25	23,25	https://www.amazon.es/ELEGOO-Microcontrolador-ATmega2560-ATmega16U2-Compatible/dp/B06Y3ZHPWC/ref=sr_1_2_sspa?__mk_es_ES=%C3%85M%C3%85%C5%BD%C3%95%C3%91&crid=16IXP8IYVEZIP&dib=eyJ2IjoiMSJ9.VNlP3CQ0NzQDPWLu2uC7KkHA__lwuDLf45_9fQSmtbWX2NOfw9qL2kbiUGF2MnDCMVOVg4_-bUWaN20Oo-pyASW_fsBa_0BKxKMcKOIPvQIcAQr6LkANQDjtDT_nyyHx2ukyr-PD484nwxI2IANpNtl_E_-62_pEWMiufkU8QHI7q1S8JpvgGJMgdeHwqG-1ufXxIfMmGLtVCMEVGeAfOb1MSvMF3AFAV1wK7aIIIRbpWm8kf6G-NnivmR22WzIv0Qw8t_W8qhw9jDU8I6Lsm2-z2Id8eIaxHEbcPxL0E-4.c8KUwVEiJ7T0gRfOboB311pEUyYhsvdQTwCAj5wkmN4&dib_tag=se&keywords=arduino+mega+con+pantalla&qid=1749642004&sprefix=arduino+mega+con+pantalla%2Caps%2C90&sr=8-2-spons&sp_csd=d2lkZ2V0TmFtZT1zcF9hdGY&psc=1
Screen 3,5’’	1	18,99	18,99	https://www.amazon.es/Binghe-Bol%C3%ADgrafo-Contacto-Resoluci%C3%B3n-Compatible/dp/B0D6B9M4ZH/ref=sr_1_4?__mk_es_ES=%C3%85M%C3%85%C5%BD%C3%95%C3%91&crid=16IXP8IYVEZIP&dib=eyJ2IjoiMSJ9.VNlP3CQ0NzQDPWLu2uC7KkHA__lwuDLf45_9fQSmtbWX2NOfw9qL2kbiUGF2MnDCMVOVg4_-bUWaN20Oo-pyASW_fsBa_0BKxKMcKOIPvQIcAQr6LkANQDjtDT_nyyHx2ukyr-PD484nwxI2IANpNtl_E_-62_pEWMiufkU8QHI7q1S8JpvgGJMgdeHwqG-1ufXxIfMmGLtVCMEVGeAfOb1MSvMF3AFAV1wK7aIIIRbpWm8kf6G-NnivmR22WzIv0Qw8t_W8qhw9jDU8I6Lsm2-z2Id8eIaxHEbcPxL0E-4.c8KUwVEiJ7T0gRfOboB311pEUyYhsvdQTwCAj5wkmN4&dib_tag=se&keywords=arduino+mega+con+pantalla&qid=1749642106&sprefix=arduino+mega+con+pantalla%2Caps%2C90&sr=8-4
Chamber polymethyl methacrylate	2,5m2	65,72(m2)	164,30	https://planchasdeplastico.es/producto/policarbonato-transparente-3-mm/?gad_source=1&gad_campaignid=16996510449&gbraid=0AAAAAoVwwA-2JSUGanHN_CR0-AZc1Ivjj&gclid=Cj0KCQjw0qTCBhCmARIsAAj8C4Y5dcppkkNshgAS0GoqejuNiEiCO95D5VttoEFx4nHkqewEDAKf7SoaAnJJEALw_wcB
Rack	3	11,00	33,00	https://www.amazon.es/Mallard-Ferri%C3%A8re-cromado-rejilla-60/dp/B00BMKWP5U/ref=sr_1_7?__mk_es_ES=%C3%85M%C3%85%C5%BD%C3%95%C3%91&crid=2V6WL13VKL0ZY&dib=eyJ2IjoiMSJ9.D1aLnzF4G36EJHClT1wbHuzBq6CHue7ZO_5WTr09IPf_FcX3ORDatQU4OY5709laVOA0idmW5NpqLC6RQz7T87oYfxxrDxjSR5eTrB0yLd1bjZbrDPnmBd7xqRa9mAxg3pcdKfTQlQGtVIfaI4jVlV9tNLabpyse-1ln13VbL5By94Qt2BkDkJo4coagITwtE3pNG-Drcpuch8YyzA4YgWY2huacB4vj4O4oZJ7au3IjG-Y1rjsIMG4AVEJtxHMyniUypJ0bWs_USfhGiPeVA_JXeFG7aufLo6DtSsklo-c.9nLvIyT4-DDJM9Y6EGfGY1Slq_SyAkWfgoQLRpgNxXE&dib_tag=se&keywords=rejilla+60x50&qid=1749639549&sprefix=rejilla+60x50%2Caps%2C83&sr=8-7
Pump 30l/min	1	61,18	61,18	https://www.amazon.es/Hailea-bomba-minuto-incluye-hidrop%C3%B3nico/dp/B00NSOQZO0/ref=sr_1_6?__mk_es_ES=%C3%85M%C3%85%C5%BD%C3%95%C3%91&crid=2QV3ZCZZQW2EP&dib=eyJ2IjoiMSJ9.8EvrXsj1Rve6ppqRPADz0-VaZ0Vpe3Ad9jM2YO1LJHfGVrsnI4xbu3vmb0839HdqjEGE8U7JU9FCOhZENWH55rtt5RVu7eWzqa-n7OEE70Ot9CG2JJupy_W4fQCEEgj1ojhxiMCgqONskinhanDqchDC4PTigzll2OZqpo_y1HUKPAySq8f8n4WtXCsYTqWiHs4TX7fCgu7U6xTNvHZS8mB-U7dgI-JvHv9Z9ODIOE6h5fac7dldxlGhVDIBLOF2mTGdx8rXOzodLoEyiE1r_xtG-xHwmzzBHrQsUgGERRc.QgNQ_J8WkHaYqVuGzuyWAp5TwTee0tu

				bDkAzO8SgjEs&dib_tag=se&keywords=bomba+60l%2Fmin&qid=1749639830&sprefix=bomba+60l%2Fmin%2Caps%2C94&sr=8-6
Pump 60l/min	1	110,00	110,00	https://www.amazon.es/AquaForte-aluminio-Silenciosa-Capacidad-regulable/dp/B006SYHCI0/ref=sr_1_5?__mk_es_ES=%C3%85M%C3%85%C5%BD%C3%95%C3%91&crid=1Z9OHLAOKFU5L&dib=eyJ2IjoiMSJ9.NRkweEtlZU8--IlxdeJnBVnA57aO3bZv5hAEsbZEYcl9xBXcXFNMVAeKlU_86n4GJzWpJ4OIB5EVtu1BudyX06OpzDGO0m-QAcaWxxlnw97ClZDvWj0Q6BCLHxdqOjyrizC-5bgqUfTjGLc7AgJx9N7xc5UT6tWYHqJTycqoAMjZkBHAgosIT_-LUFZez5Z_CAMOPlR3IbjEVCJU9N_bvnvFdB68xiLwvSfY2hTUrhiUBE75D232bex3DA3G01LUZhVONH23IfRAz2U_0am5jKcT0EFB2WKabKOpAJOVNUM.SmnSqG7K3XQG0_bTTbTVbFH7hzXOCEkY8uRXlZar1lc&dib_tag=se&keywords=Bomba%2Bv30&qid=1749640500&sprefix=bomba%2Bv30%2Caps%2C130&sr=8-5&th=1
3D printing	-	31,00	31,00	https://es.farnell.com/ultimaker/1609/filament-pla-black-750g/dp/2992628?gross_price=true&CMP=KNC-GES-GEN-SHOPPING-Pmax-High_ROAS&gad_source=1&gad_campaignid=18071281895&gbraid=0AAAAAD8yeHlKxiGMy79WCfJwNBT_BsqP9&gclid=Cj0KCQjw0qTCBhCmARIsAAj8C4b-IsF7T5oIuiyewkZV-nNr8tDFVX0Vy5St76HWuHN5j-02Z0rQICEaAhJBEALw_wcB
Thermoelectric cooler	1	47,41	47,41	https://www.amazon.es/Refrigerador-Refrigeraci%C3%B3n-Termoel%C3%A9ctrico-Enfriamiento-Semiconductores/dp/B0F6NWNWJM/ref=sr_1_16?__mk_es_ES=%C3%85M%C3%85%C5%BD%C3%95%C3%91&crid=1XPI9W7G6FJMY&dib=eyJ2IjoiMSJ9.9lY2M3EO0bKYlISgJaOjbFR0CO1MNnuPKF7FQmBmf24793Shg3oHhO-N_JNhPTuMwtWic163ggGxBsgEaRs6K16S-PeHerDs4niKZfdYLnI9DrVWBIBR1iV60ukXPu3hhjJTvsIpVfQ1XD-gxILKkw2GAAQosMOAUKuB3SloCD-4lxuFh2BLEdseAl5S91JsiijAaGn6LtfQBilioHr3EaD2cnSsogdszxBCcni6g-tGlKlw5CnGXptQwrq8BEX4UCFTLGcdRjignZ0kLzgellU7xlUn5pjFYNRXYykJEW0.kDaw92Wqmkk6qb-3Jdq5KTzDpfY6PgVISbfYPW0V8aU&dib_tag=se&keywords=peltier+12v+120W+liquido&qid=1749724430&sprefix=peltier+12v+120w+liquido%2Caps%2C89&sr=8-16
Water cooling radiator	1	15,99	15,99	https://www.amazon.es/CENPEK-Radiador-refrigeraci%C3%B3n-intercambiador-computadora/dp/B09FXL787S/ref=sr_1_12?__mk_es_ES=%C3%85M%C3%85%C5%BD%C3%95%C3%91&crid=PQGXM2YHEEV3&dib=eyJ2IjoiMSJ9.yjbbkS_YR53lsFDVB9rz0eYd5sZpphUjuTHx0GHaUkYI5UlNnAXcYGrK5NPn7pJfDK7bzqOrTpZhn_NPIxlIyE2goUsz_bcNBKzDvYGkaIZlo9KPsHoiczEU67CN8_tpfdQOO35OF_DK9ZAaofcYhHLr3qrTsUEJqPhkwz7V0Bg8SO2REwV15OTBLg_uwcM5tloSxGjuGmAhrcS_X7Qrxw18W9LjGTun564uKLiel05dAQiT5TZ6nAGbaw8igOMGZmzpsWvEZrDNOEXwrxasGjp5itvNilLNFstgyNaFOJc.G1-K8oQzQAXi-uTpVrxW7Fyd0eNC0x3PsQHYMwMlq-4&dib_tag=se&keywords=radiador+liquido&qid=1749724514&sprefix=radiador+liquido%2Caps%2C77&sr=8-12
Water cooling pump	1	19,72	19,72	https://www.amazon.es/Diyeeni-refrigeraci%C3%B3n-expansi%C3%B3n-m%C3%A1x-Bomba-enfriamiento/dp/B07ZRQRV7J/ref=sr_1_4?__mk_es_ES=%C3%85M%C3%85%C5%BD%C3%95%C3%91&crid=2PGY6ZH64SWIS&dib=eyJ2IjoiMSJ9.fLA9IrZ-4xIiJiCZDNLIo0iOr0srTdzEtk26K6yuy7gC27QZgzKO3yCZpTJPTes98E8sfvWLDY58QdMIxf7WFjlln4dTfSQemugONaQwo8cqYc70UEWTb35qg50KeciM-G5VzBf4mZiaAk483FHoIRd8rsp9fGP9PY_fHRqzVkEE9MAFjzffeJJapV2YuOewTKz0QHwzmhtHavNzgWzUONq1C4nojCNiIV_by89kzD9YEq5_OL82FUaG-W6l5QxrdNt7rga4DEEUKZW26d28iO6IUbgxF22iBQ_jiEh9h6o.T2DoqO2ThfgHihFKC9f4I2e8n3kWlOl2-tvC-n82Zew&dib_tag=se&keywords=bomba+liquido+refrigeracion&qid=1749724631&sprefix=bomba+liquido+refrigeracion%2Caps%2C77&sr=8-4

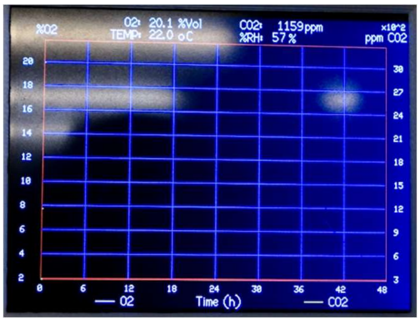
The total cost of the materials for building the device is 633,77€. Materials such as resistors, LEDs, pin connectors, capacitors, PCB, ICs, and fuse holders were purchased as a kit, however, not all materials available in the set were used when building a single device. Most of them can be also easily reused from obsolete/damaged consumer electronic devices or household appliances.

4. Build instructions

5.1. *3D Design and Printing*

The chamber was built using polymethylmethacrylate sheets, forming a sealed enclosure with three hinged doors that allow the insertion of mice cages. To ensure proper closure, a metal rod is used to apply pressure on each door against the chamber frame. Custom 3D-printed holders were designed and fabricated to hold the metal rods securely in place. On the side of the chamber, additional 3D-printed holders are used as storage for the metal rods when they are not in use. Beyond structural mounting, 3D printing was also leveraged to develop custom protective enclosures for the gas sensors affixed to the chamber. These cases were specifically designed with dedicated slots and openings for efficient gas exchange. A custom 3D-printed case was also created to house the electronic circuit incorporating the Arduino board. This enclosure includes ventilation slots to prevent overheating, a front opening for the display module that visualizes real-time data and system graphics, and a rear connector interface for power supply integration. 3D printing was also employed to create accessory components for the soda lime containers. These components ensure optimal airflow for efficient CO_2 removal. Furthermore, a funnel specifically designed for refilling the soda lime containers was also produced using a 3D printer. Figure 3 shows the 3D-printed components.

a)



b)

c)

Figure 3: 3D-printed components, including the sensor enclosures (a), the Arduino and electronics housing (b), and the metal rod supports used for sealing the chamber doors (c).

5.2. *Electronics*

The electronic system, shown in figure 4, has been designed to interface the gas sensors with a microcontroller unit (MCU), specifically an Arduino Mega, while also enabling control of electromechanical actuators such as a solenoid valve and pumps via a transistor switch and a solid-state relay, respectively. An I²C-based Sensirion SCD4x sensor is employed for measuring CO_2 concentration, temperature, and humidity, while oxygen levels are monitored using a DFRobot I²C oxygen sensor module. The schematic is organized into distinct functional blocks: sensor interfacing, power regulation, and actuator control. The system operates from a single 12V DC power source. Two linear voltage (7805 for 5V and 7809 for 9V) regulators are used to derive the required supply voltages for the other components.

The injection of N_2 into the chamber is allowed by a solenoid valve driven by TIP122 NPN Darlington transistor that acts as a switch.. A flyback diode (1N4148) is placed in parallel with the solenoid coil to protect the transistor from voltage spikes. This valve remains closed except when activated. The introduction of room air through the pump is controlled via a digitally-activated solid-state relay, which provides electrical isolation and long-term durability in switching operations.

To regulate humidity inside the chamber, a cooling system based on a Peltier cell is used. This system consists of a thermoelectric cooler, a radiator, and a fan. The Peltier cell cools the air circulating through the chamber. As the air cools, it condenses on the cold surfaces inside the dryer. This process effectively removes the water vapor produced by the mice, which helps maintain a stable relative humidity. The cooled and dehumidified air is then returned to the chamber.

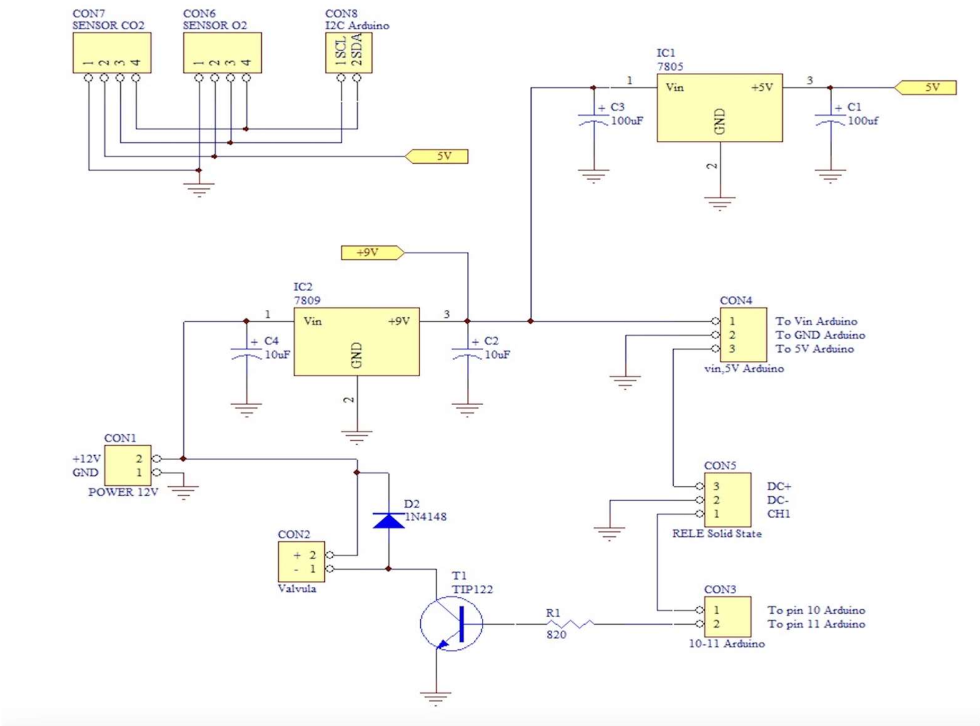


Figure 4: Schematic

5.5. ***Arduino control***

The Arduino firmware is structured to manage real-time acquisition, display, control, and recording of oxygen (O_2) and carbon dioxide (CO_2) concentrations, along with temperature and relative humidity. These values are periodically sampled and visualized on a 3.5" TFT LCD driven by the TFT_HX8357 library. Upon initialization, communication with all peripherals is established via I²C and SPI interfaces. A graphical interface is then rendered on the display, including axes for time and gas concentration, grid lines, and fixed legends for the visual interpretation of data. A rectangular plotting area is drawn for graphing real-time measurements. Initial error checks are performed to verify sensor integrity. The main function of the code contains the core operational logic and it is executed iteratively. At each iteration, the system checks whether new data is available from the CO_2 sensor. If any error occurs, corrective actions are taken: the nitrogen solenoid valve is closed and the air pump is activated. Once valid data are retrieved, the CO_2 concentration, temperature, and humidity values are updated. Simultaneously, the O_2 sensor performs an averaged measurement based on ten samples, providing a smoothed value of the ambient oxygen concentration. The numerical values of humidity, temperature, CO_2, and O_2 are updated on the display interface every 3–5 seconds, in accordance with the conversion time of the CO_2 and O_2 sensors. Approximately every 7 minutes, the system plots instantaneous values of O_2 and CO_2 on a dynamic scrolling graph. The X-axis represents time (scaled to cover 48 hours with 412 data points), while the Y1 and Y2 axes are scaled to appropriately display O_2 (%) and CO_2 (ppm) concentrations, respectively. A circular buffer mechanism ensures efficient redrawing of the graph without overloading memory. Figure 5 shows the graphical interface on the display. Simultaneously, the system logs environmental data to an SD card every 60 seconds, including timestamp, temperature, humidity, O_2 concentration, and CO_2 concentration. This allows for offline analysis and long-term monitoring.

The control system is managed by two feedback routines. On the one hand, if the oxygen concentration exceeds a predefined setpoint (11%), the system activates the N_2 valve until the level reaches the setpoint. Once this value is reached, the valve is deactivated, cutting off the N_2 supply and maintaining the chamber's O_2 level close to the desired value. Over time, as O_2 concentration increases again, the N_2 valve is reactivated, initiating a new control cycle. On the other hand, if the O_2 level drops below a critical setpoint (10.5%), the N_2 valve is automatically closed, and a room air (i.e., oxygenation) air pump is activated until the O_2 level is restored to 11%. Additionally, if the CO_2 concentration exceeds a critical threshold (5000 ppm), the air pump is also activated. It remains on until the CO_2 level falls below 2500 ppm.

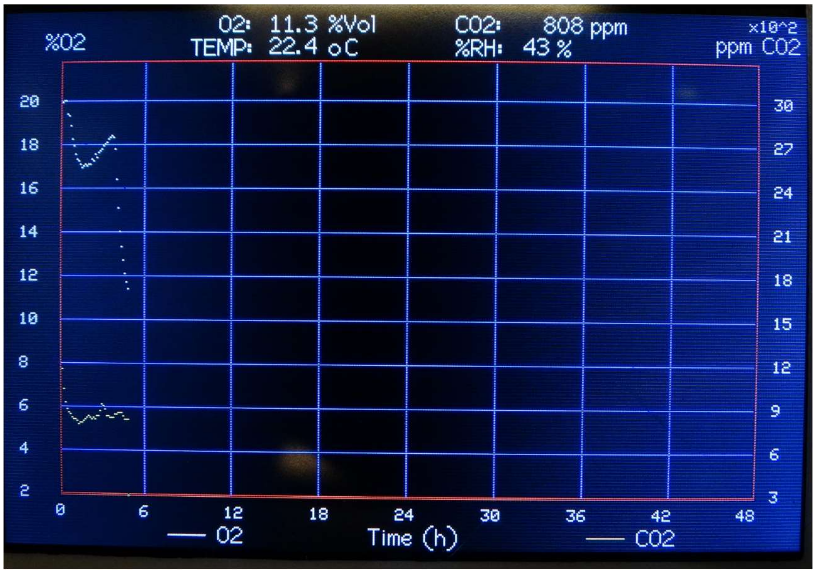


Figure 5: The graphical interface on the display. This example shows the first 5 h of a recording where the N_2 source functioning was intentionally altered to more clearly observe sudden changes in O_2 concentration.

5. Operation instructions

Starting application of chronic hypoxia to mice requires the following initial actions: connecting the device to an electrical power line (preferably with backup), placing a recipient at the outlet tube of the Peltier-based air conditioning unit to collect the condensed water, filling the soda lime containers, connecting the device N_2 inlet to a pressured source (e.g, a cylinder), setting its low pressure/flow regulator to provide a N_2 flow able to reduce the chamber O_2 concentration from room air (21%) to the 11% set point in 10-20 min, place the animal cages into the chamber, carefully closing the windows and pressing the power on button.

During application of continuous hypoxia, the real time values of O_2 and CO_2 concentrations, temperature and relative humidity within the chamber can be seen in the front panel of the control unit. The screen in the front panel shows an updated time course of O_2 and CO_2 concentrations for the last 48 h (e.g., allowing checking that the system has worked correctly along an unattended weekend).The most important maintenance task is to replace the soda lime when required (either because it starts changing color form white to blue or because CO_2 concentration starts to increase above desired values). When a mice cage is extracted from the chamber (either for cleaning, feeding replacement or for animal examination), the window must be closed immediately to minimize gas concentration changes into the chamber.

6. Validation and characterization

The *in vivo* performance of the device was validated when applying it in research studies where mice were experiencing chronic hypoxia. Figure 6 shows an example of the O_2 and CO_2

concentrations, temperature and relative humidity signals recorded when 17 wild-type mice were subjected to 11% O_2. Data in the figure starts when the mice were initially introduced into the hypoxia chamber at conventional lab ambient conditions and hypoxia application was initiated. As expected, O_2 concentration decreased from lab conditions (21%) to the 11% set point and subsequently remained stable at this value with negligible oscillations (10.8–11.2 %). CO_2 absorption was very effective since its concentration was reduced from the initial room lab value (≈1000 ppm; i.e. 0.1%) to ≈800 ppm, showing a considerable reserve capacity of the device to keep safe levels of CO_2 concentration. The figure also shows that, after a very minor initial fluctuation resulting from the sudden N_2 injection to lower O_2 concentration until the set point, ambient temperature and relative humidity in the hypoxia chamber were maintained at values very close to the external lab room air ≈45% and ≈22 ºC, respectively. N_2 consumption to keep the 11%-O_2 state steady was only ≈2 l/min.

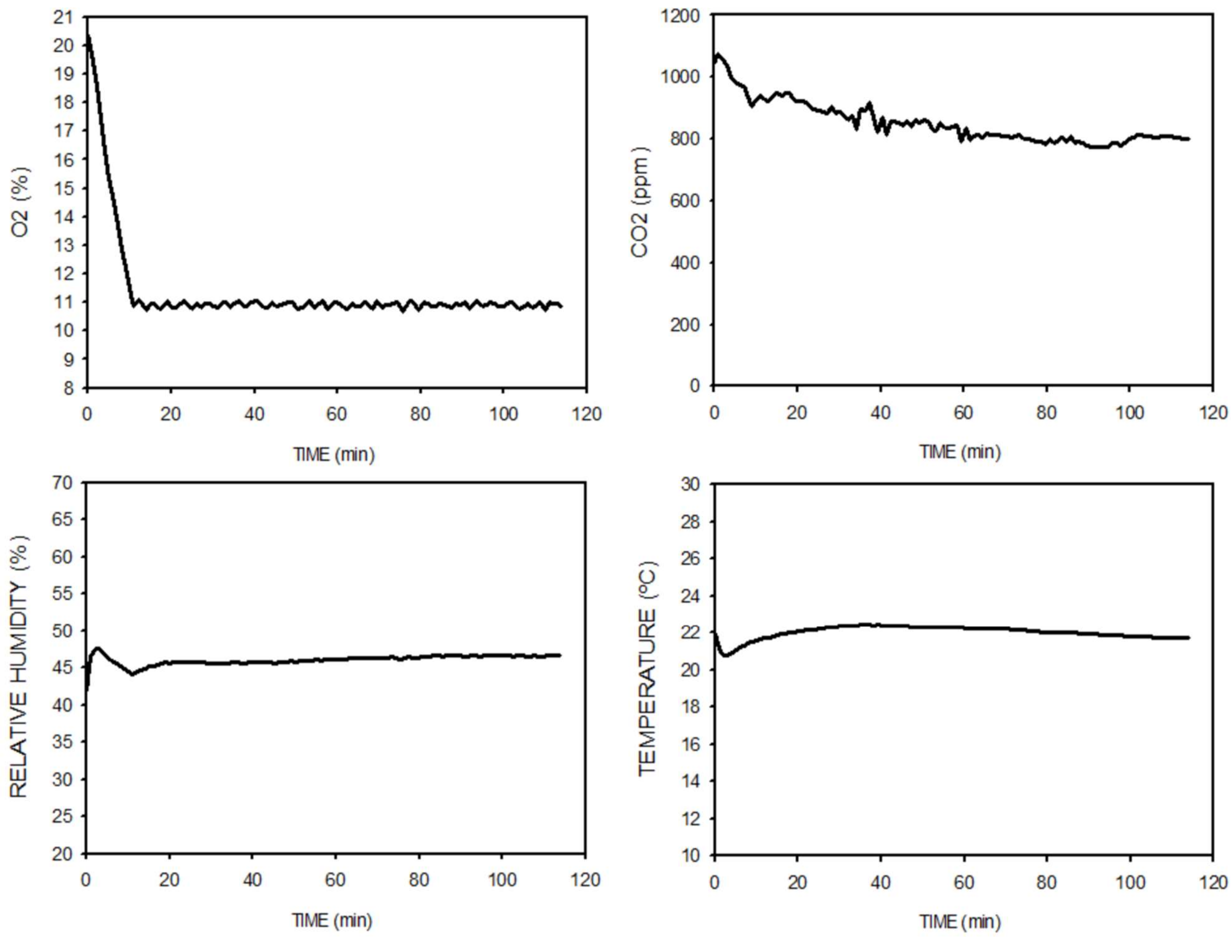


Figure 6. Example of the signals recorded inside the hypoxic chamber when subjecting mice to 11% chronic hypoxia from initial lab air conditions.

Ethics statements

The mice experimental procedure was approved by the Ethical Committee for Animal Research of the Vall d'Hebrón Research Institute of Barcelona (approval number 51/24).

CRediT author statement

Jorge Otero: Technical conceptualization, Methodology, Writing-Reviewing; **Daniel Mbanze**: Methodology; **Miguel A. Rodríguez-Lázaro**: Methodology, Validation; **Raffaella Salama**: Methodology, Writing; **Gorka Solana**: Methodology; **Vicent Muñoz-Vaño**: Animal model testing; **Yolanda Cámara**: Animal model testing; **Isaac Almendros**: Animal model testing; **Ramon Farré**: General conceptualization, Methodology, Validation, Writing-Reviewing, Editing, and Supervision.

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