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Christophe Hecquet, Maylis Etcheberry, Nadine Ravel, Philippe Litaudon, Frédéric Pain. Naturalight: A controlled illuminated environment to study light exposure impact on behavior and cognition in freely moving rats. *HardwareX*, 2025, 23, pp.e00662. <10.1016/j.ohx.2025.e00662>. <hal-05102366>

HAL Id: hal-05102366

<https://hal.science/hal-05102366v1>

Submitted on 7 Jun 2025

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HardwareX

journal homepage: www.elsevier.com/locate/ohx

Hardware Article

Naturalight: A controlled illuminated environment to study light exposure impact on behavior and cognition in freely moving rats

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ARTICLE INFO

Keywords:

Illumination dosimetry
Low light level exposure
LED
Rat
Impact of light exposure on behavior
Memory

ABSTRACT

In humans, scientific studies have shown that the increased use of screens at night and prolonged exposure to artificial light, particularly blue light, can significantly disrupt the organization of sleep-wake cycles and the functions that depend on them like memory. In rodents, moderate and even weak exposures to light have been shown to have various and either detrimental or beneficial effects on circadian rhythms, behavior, cognition. Yet, scattered and sometimes opposite effects are reported in the literature, which is largely attributed to differences in experimental conditions, especially on the definition and evaluation of the actual dose of light received. To address this issue, we have built an enclosure with controlled and characterized illumination. It can be used as a standalone experimental environment or eventually connected to naturalistic environment where several animals live constantly and interact freely within several housing modules, including the controlled illumination one.

Specifications table.

Hardware name	NATURALIGHT
Subject area	Neuroscience
Hardware type	Controlled illumination environment for behavioral studies in rats
Closest commercial analog	No commercial analog is available
Open-source license	CC-BY 4.0
Cost of hardware	About 1500 € for the mechanical enclosure and 2000 € for the controlled illumination at several wavelengths, for a total of around 3 500 €breakdown of costs is detailed in the Bill of materials file
Source file repository	https://doi.org/10.5281/zenodo.14616383

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<https://doi.org/10.1016/j.ohx.2025.e00662>

Received 2 April 2025; Received in revised form 29 May 2025; Accepted 30 May 2025

Available online 3 June 2025

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1. Hardware in context

In mammals, exposure to light, particularly blue light, has been shown to have either detrimental or beneficial effects on circadian rhythms, behavior, cognition [1–4]. These scattered and sometimes opposite effects could be largely attributed to differences in experimental conditions, especially on the definition and evaluation of the actual dose of light received. To address this issue in rodents, we have built Naturalight, an enclosure where lighting is controlled in terms of wavelength, instantaneous irradiance and total energy received. Although many studies in the literature have been conducted on the effect of light on sleep and cognition, we have found no similar available hardware either proprietary or open source. In most of the published studies, illumination is achieved with LEDs or, in few studies filtered white light or lasers [1–7]. In many studies, light exposure is poorly characterized, as only the light “color” or central wavelength are provided. Similarly, only the total light power at the source output is provided rather than the exact irradiance received at a certain distance, under a certain angle etc. It has been pointed out that the experimental illumination conditions suffer from a lack of proper calibration and lacunar description [8–9]. In Naturalight, the spectra of different high-power LEDs have been characterized. In addition, experimental measurements have been carried out to establish irradiance maps within the enclosure. Finally, understanding the effect of a specific light source implies to convolve the light spectrum with the spectral sensitivities of the rods, cones and melanopsin receptors specific to the species under study [8]. Here we propose a low cost, easy to reproduce method to build and properly characterize an illumination enclosure for behavioral experiments on freely behaving rodents. This consideration of the physiological characteristics of the species studied is in line with the recent evolution in preclinical neuroscience studies towards a more naturalistic approach to the effects of the environment. Thus, the Naturalight module was designed to be part of a larger naturalistic environment that allows rats to express most of their behavioral repertoire, in particular spatial navigation, territoriality and social interaction. This module can also be used as a temporary housing to carry out experiments in precisely controlled illumination conditions.

2. Hardware description

The Naturalight module is composed of a standard plastic box with length = 600 mm width = 400 mm height = 426 mm like those of the other living modules (length = 610 mm width = 400 mm height = 225 mm) (Fig. 1). Holes in two walls have been created and are associated to passage flanges parts fixed to the box. These allow connection through standard tunnels with other modules within the naturalistic habitat composed of eight other housing boxes adapted from commercial ground hamster housings (see Fig. 11). Standard additional accessories such as water bottles, food dispensers or behavioral, conditioning and test elements can be added to the living environment. Animals’ displacement and behavior are recorded and timed precisely using several synchronized cameras equipped with wide angle lenses.

A roof made of X-rails structure and polycarbonate walls is fixed on the top of the box. Its height is chosen to optimize the spatial homogeneity of the irradiance for the given LEDs at the height of the freely moving animals (in our case a LED-floor distance of about 60 cm was chosen, see Fig. 2). A removable camera and LEDs panel is fixed on top of the roof. The box walls are spray painted with a

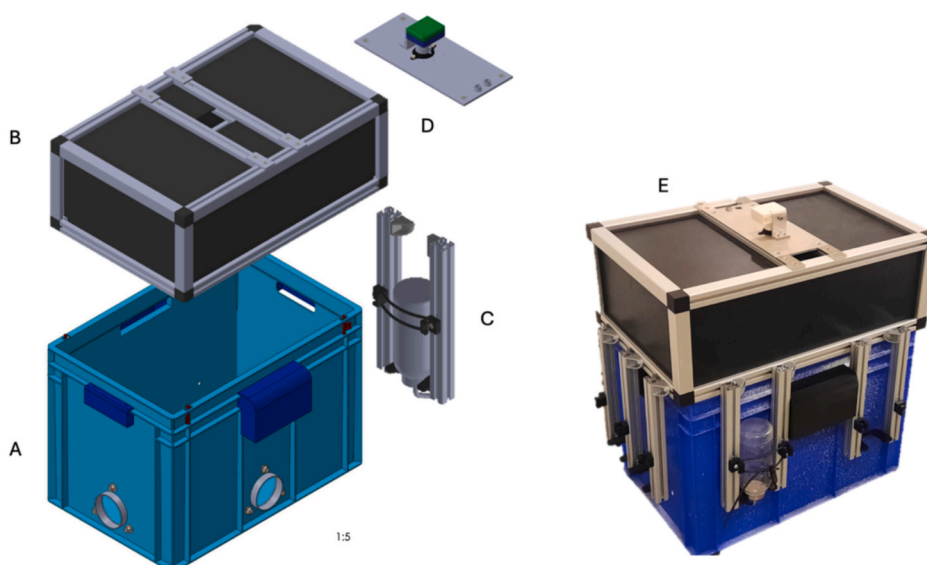


Fig. 1. Naturalight enclosure. A) Box with round passages flanges for connection to other modules, 3D printed light tight caps (dark blue). B) Roof made of X-rails structure and polycarbonate sheets walls. C) Bottle holder made of X-Rails, printed parts and O-rings D) Led and camera panel to be fixed in the central part of the roof. Details of dimensions and part lists are given as mechanical diagrams in naturalight_all.pdf file. E) Photograph of the complete device.

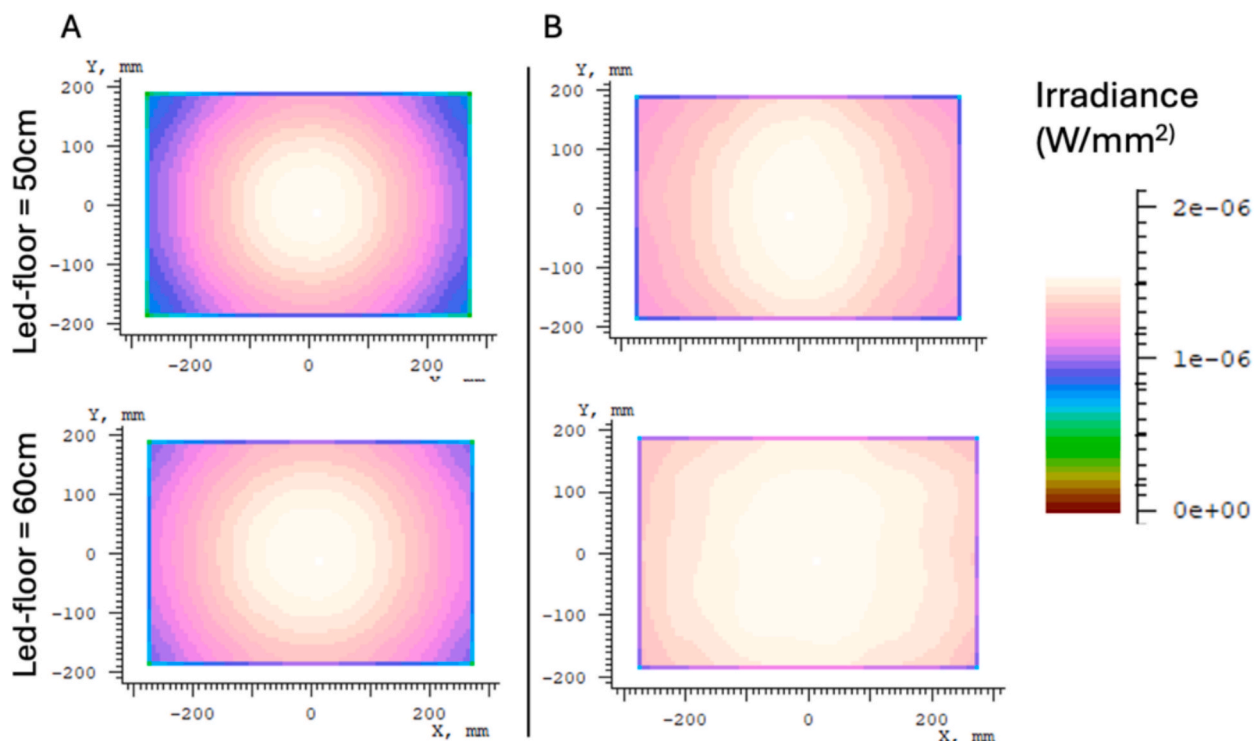


Fig. 2. Effect of LED-floor distance and diffusive paint on the irradiance homogeneity at the floor level for a LED with a 80° emission angle. A) Without diffusive paint B) With diffusive paint. Simulations carried out with Lighttools Illumination software [10].

diffusive paint with isotropic diffusion to optimize the spatial homogeneity of illumination. The whole environment is equipped with cameras to record the movements, poses, interactions of the animals which are further analyzed using either manual or automated analysis.

In a research laboratory context, Naturalight can be used to:

- Study the impact of light exposure on sleep, memory and learning, stress, food intake in living non-anesthetized rodents undergoing social interactions.
- Investigate a vast range of parameters including irradiance level, effect of spectral composition, exposure duration, exposure time of the day, exposure frequency.
- Outside of its original purpose, the enclosure with well-controlled irradiance level and spectrum could be useful for studies implying light exposure of any object, living or not.

3. Design files summary

All the design files listed in the table below are available on Zenodo [9].

Design file name	File type	Open-source license	Location of the file
<i>bill_of_materials.ods</i>	spreadsheet	CC-BY	https://doi.org/10.5281/zenodo.14616383
<i>naturalight_all.pdf</i>	3D figures	CC-BY	https://doi.org/10.5281/zenodo.14616383
<i>mechanical_files_Solidworks.zip</i>	CAD files	CC-BY	https://doi.org/10.5281/zenodo.14616383
<i>mechanical_files_STEP.zip</i>	CAD files	CC-BY	https://doi.org/10.5281/zenodo.14616383
<i>mechanical_files_STL.zip</i>	3D printer files	CC-BY	https://doi.org/10.5281/zenodo.14616383
<i>elec_cable_connection.pdf</i>	Electrical scheme	CC-BY	https://doi.org/10.5281/zenodo.14616383

The *bill_of_materials.ods* file contains all the parts required and the tools used to build and characterize the device. *Naturalight_all.pdf* shows 3D views of the system. The first two pages correspond to global views and each other page shows the different elements and arrangements of each sub-component (box, roof, water bottles fixing parts, LED and camera panel). The archive file *mechanical_files_Solidworks.zip* contains all the mechanical parts and assemblies of the project in the Solidworks file tree structure. As Solidworks is a proprietary format, we provide alternatively the corresponding mechanical files in *mechanical_files_STL.zip*. It contains the mechanical parts as generic step files that can be opened and modified in any CAD software. The zip file *mechanical_files_STL.zip* contains

the design files of the 3D printed parts listed in the bills of material (box light covers and camera light trap parts, closing brackets and bottle holder fixing jumpers parts). Finally, elec_cable_connection.pdf shows the electrical connections between the LEDs and the LED controllers.

4. Bill of materials summary

The bill of material is available in the bill_of_materials.ods file in the Zenodo repository of the project. The items corresponding to each of the sub-components (box, roof, camera and led panel) are listed along with the tools used to build and characterize the lighting-controlled enclosure.

5. Build instructions

The starting point for building the system is the file naturalight_all.pdf. It shows a global view of the Naturalight modules and specific views of each sub-components. Each sub-components page has a list of the parts used. In mechanical_files_Solidworks.zip there is a separate file for each subcomponent with these parts referenced with the same reference names. Files are organized according to Solidworks files tree structure such that there is access to the details for each part within each general structure files. All 3D prints files are located with self-explanatory names in the mechanical_files_STL.zip file.

Box construction

The box is shown as box in the naturalight_all.pdf file and named accordingly “box” in the bill of materials. It is standard plastic box modified to create two junction holes compatible with the other modules. Two passage flanges are attached using M8 nuts and bolts to these holes for tunnel connection with two other living modules within the naturalistic environment. The design files of the flange parts are available in the mechanical_files_Solidworks.zip file as well as in the alternative mechanical_files_STEP.zip file. Holes in the top of the box are drilled to position two or more 3D printed brackets used to attach the roof to the box. Depending on the set-up, additional drilled holes can be used to fix the water bottles using the 3D printed bottle holder and fixing jumpers. Water bottles are then held with O-rings on these supports. Finally, four 3D printed light covers (two small and two big ones) are attached to the handles holes of the box to make it light tight, while preserving air venting. The corresponding 3D print files are all available in the STL.zip file.

A) Box walls and ceiling painting with a diffusive paint.

A-1 Simulations of LED illumination

Simulations with a commercial illumination software [10] have shown that covering the walls with a light diffusive paint with a Lambertian profile significantly improves the homogeneity of irradiance between the sides and the center of the enclosure floor. Fig. 2 shows representative simulations of the irradiance maps at the box floor level for a single LED with an emission angle of 80° placed at the center of the roof on the adapter plate. We have considered two different difference heights relative to the box floor. Increasing the distance between the floor and the LED slightly improves the homogeneity of the illumination at the floor level. In addition, the diffusive paint on the walls strongly increases the irradiance map homogeneity, especially on the sides of the box. For a LED-floor distance of 60 cm with painted walls the relative difference of the irradiance between the center and the sides is less than 10%.

A-2 Walls and roof painting procedure.

Practically, a spray ready emulsion of BASO4 powder in water (ODP97, GigaHertz Optik, Germany) was chosen as it is a highly diffuse optical coating with typical reflectance of 97 % in the 380–780 nm spectrum. The material is non-toxic, and free of solvents. First, to ensure the best possible adhesion of the paint to the plastic box walls, they are lightly sanded and then washed and degreased

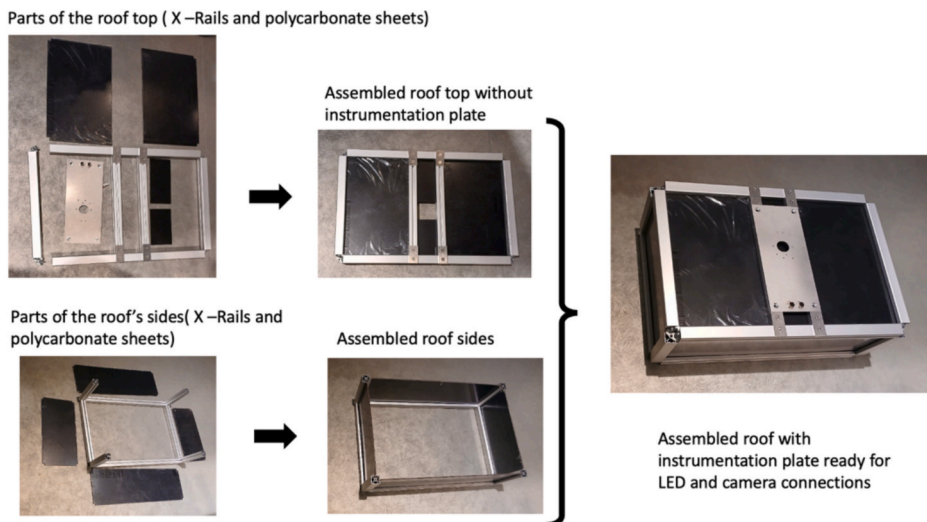


Fig. 3. Building steps of the X-rail light-tight roof structure.

with a hand dishwashing detergent. After the walls have dried, the paint is applied with a spray gun with a nozzle of 2.5 mm diameter operated at a 2.5 bar pressure. A viscosity cup is provided with the paint spray gun to check adequate viscosity using the cup emptying time method. Depending on the spray gun, the viscosity of the paint may have to be adjusted by a slight dilution with pure water. The coating is applied in successive layers with increasing thickness starting with a very thin layer and increasing the drying time between layers (2 min, 3 min 6 min, 10 min, 15 min, 20 min 27 min, 30 min, 40 min). The delays are indicative minima, and the general rule is that the previous layer must still be damp (not completely dry) when applying the next one. At this point, the almost dried painting loses its gloss and becomes more or less matte. In our experience 7 layers are far enough to obtain a homogeneous cover of the walls (acceptable results can be obtained with 4–5 layers depending on the skills of the painter). The nozzle of the paint gun is initially set to its minimum to provide a very thin layer and it is increased gradually (about $\frac{1}{4}$ turn of the nozzle between each layer for the paint gun we used). Paint should be applied in a clean, ventilated room while wearing protective gloves and mask. The paint spray should be perpendicular to the surface and located about 50 cm away from it to ensure homogeneous layers. As the paint is relatively sensitive to scratches, we have finally sprayed a double layer of pastel painting fixative. This allows avoiding scratches and permits manual cleaning and disinfection of the enclosure with veterinary approved detergents.

B) Roof construction

B-1 X-rail structure.

The top of the box is composed of a *roof*, which is a cuboid structure made of x-rails (Fig. 3). The structure was acquired as a kit from the manufacturer (elcom, France), but any X-rails structure with associated nuts, bolts and connectors can be used. The roof is attached to the box with 3D-printed brackets. The X-rails structure base is rectangular with the same width and length as the plastic box. It is build using standard X-rails, X-rails corner cubes connectors and sliding nuts and bolts.

B-2 Light-tight tricks.

Caution was taken to avoid as much as possible light leaks from the outside into the box. Therefore, whenever possible, we used X-rails with a minimal number of grooves. Notably, a specific part is used to cover the corners of the roof to keep them light tight (It can be seen on Fig. 2E). The walls of the structure are composed of polycarbonate sheets. As the sheets are thinner than the X-rail grooves, additional black joint is used to ensure light tightness. Alternatively, black cardboard such as TB4 black cardboard (from Thorlabs) instead of polymer sheets could be used to decrease the total weight of the structure and ease its handling.

B-3 Advice on the choice of LED for homogeneous illumination

To simplify the illumination scheme, a single high-power LED is used for each required wavelength. To set the optimal distance between the LED and floor the vertical X-Rails of the roof were chosen to be 17 cm long resulting in a total height of 62 cm between the LED and the floor of the box. We advise using LEDs with the largest emission angle as possible to favor illumination homogeneity. LEDs with angular emission smaller than 80° should be avoided if possible. However, the distance between the LED and the box floor could be increased to maintain acceptable irradiance homogeneity. To facilitate generalization of the set-up for any available LED, we provide in Table 1, the advised distance between LED emitter and floor as a function of the angular emission to obtain homogeneous illumination at the floor level.

C) Water bottles option

Optionally, a water bottle holder can be attached to one of the X-rail and the water bottles set with O-ring on their support. The parts and general plan for the bottle holders are shown on Fig. 4.

D) LEDs and camera plate construction

D-1 LED and camera Plate description.

The *LEDs and camera plate* is attached with screws to the roof. A photograph of the plate is shown on Fig. 3 without the LEDs and camera attached. Details of the equipped plate are shown on Fig. 5 and the corresponding mechanical design file is available in the `mechanical_files_Solidworks.zip` file or alternatively in the `mechanical_files_STEP.zip` file.

The plate bears two LEDs on PCB at two different wavelengths (see Fig. 5 D). We used high power LEDs on PCB from Thorlabs with manufacturer peaks at 375 nm and 450 nm. (We also list other references easy to substitute with other wavelengths in the `bills_of_material.ods` file). Having two different LEDs on a single plate allows conducting studies involving two different wavelengths without having to modify the set-up. The LEDs are positioned very close, at the middle of the plate to ensure illumination homogeneity.

D-2 LEDs control.

General cabling.

Two electrical connectors are attached to the plate, one for each LED (parts#8 on Fig. 5). For each LED on PCB, a pin is soldered to each ‘-’ and ‘+’ pad, with 0.1 in. (2.54 mm) spacing between the 2 pins. Then, each LED PCB is set with 2 M2 screws and thermal paste to the instrumentation plate. Each LED is then connected to its power supply as shown in the `elec_cable_connection.pdf` file). Either a commercial system (Thorlabs LEDD1B) or a low-cost LED driver (Low-cost supply alternative listed in the `bills_of_material` file) can be used to provide adequate and adjustable current and software control through external trigger or analog modulation.

Commercial LED Driver.

Table 1

Advised LED emitter to floor distance for homogeneous illumination at the animal level.

LED Angular emission (full angle in $^\circ$)	Minimal advised distance between LED emitter and floor (cm)
60	90
80	60
120	50

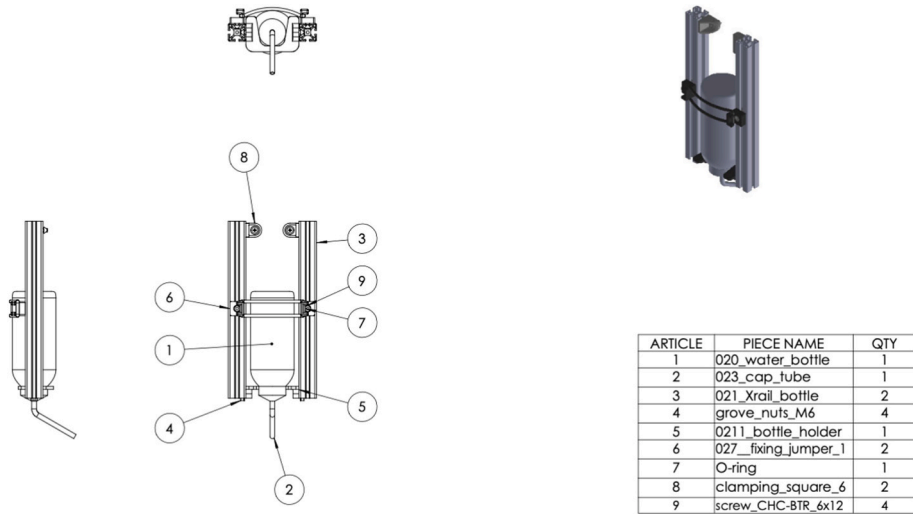


Fig. 4. Plans for the bottle holders.

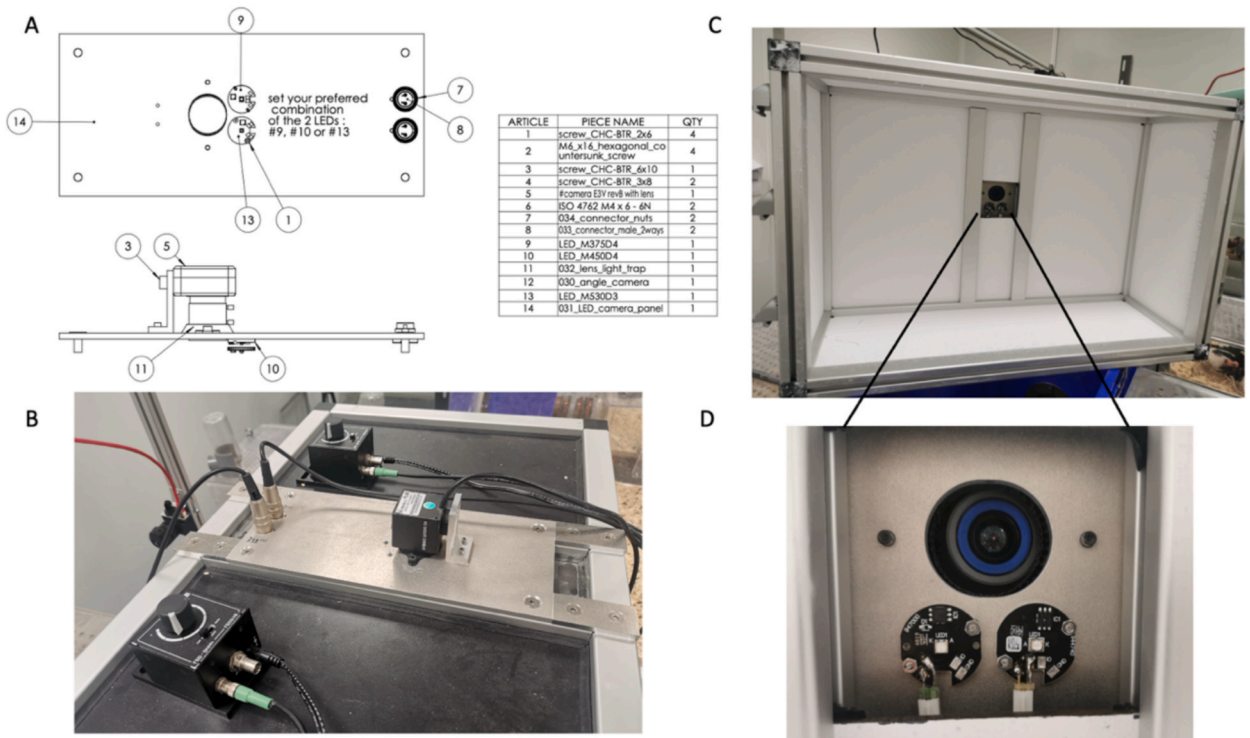


Fig. 5. A) Plans from the bottom and side of the LED and camera plate. B) Photograph of the LED and camera plate with 2 commercial LED drivers and the camera connected C) View from the inside of the roof after painting D) Zoom on the camera lens (top) and two LEDs on PCB (bottom).

For the commercial LED driver (LEDD1B), a three-position slider allows to choose between manual, TTL ON/OFF or analog modulated control (Fig. 6 A). For the manual position (labeled CW) the power of the LED is simply set using a manual knob while for the other two positions a BNC terminated cable can be connected to provide either a TTL (TRIG position) or analogous modulation (MOD). The TRIG and MOD options allow to control the system remotely thus avoiding disturbing the animals and reducing this source of potential behavioral bias. A current limit (up to 1,2 A) can also be set to avoid driving the LED with current above its specifications or to conveniently set a maximal admitted irradiance. We have used this system in CW mode with a current limit of 1.2A. Fig. 6 B shows the irradiance measured at the center of the Naturalight enclosure for different knob position for two different LEDs. Notably, the graphs are not linear, underlining that calibration is required to ensure proper quantitation of light exposure.

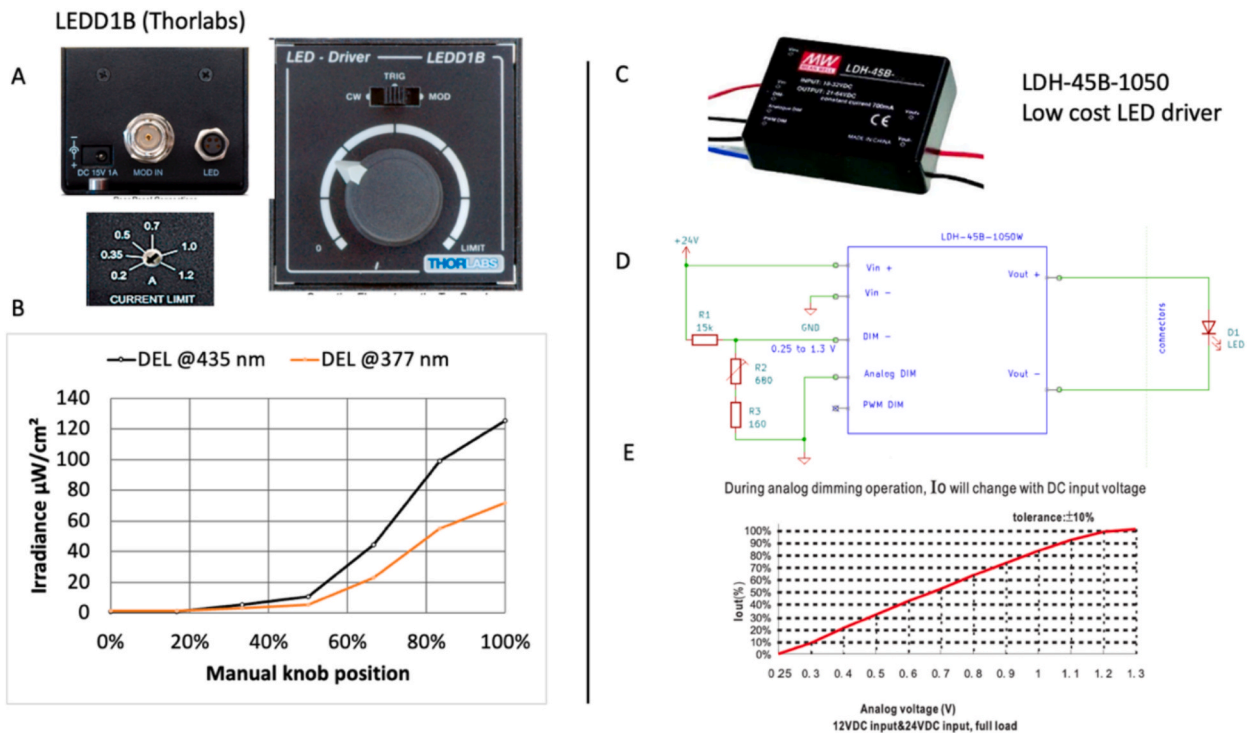


Fig. 6. LED driving and control options. A) Commercial LED driver with manual, TTL (ON/OFF) and modulation options. Current limitation allows protection of LEDs. B) Experimental measurements of the irradiance at the center of the Naturalight enclosure for different position of the knob in manual control. C) Low-cost LED driver D) Electrical scheme for analogous modulation of LDH-45B-1050 Led Driver E) Manufacturer specifications of the linear relationship between analogous modulation voltage and output LED driving current.

Low-cost LED Driver.

Alternatively, a low-cost LD-45B-1050 LED driver (about 1/15 of the cost of LEDD1B) can be used (Fig. 6 C). The electrical scheme for analog control of LED driving current is shown on Fig. 6 D. A 0.25 to 1.3 V DC voltage applied between DIM- and Analog DIM pins drives the LED with a current between 0.25 and 1.2 A. According to the manufacturer specifications, the driving current is proportional to the analogous voltage with a +/-10 % tolerance.

D-3 Camera.

The camera and its lens are positioned at the center of the plate pointing toward the floor through a hole in the plate (see Fig. 5 D). A 3D printed *lens light trap* is secured with two screws around the lens (blue part on Fig. 5 D), again to ensure light tightness. Then, the camera is attached to the 3D printed camera bracket with one screw. An RJ45 Ethernet cable is used to connect the camera to the associated driving and recording system.

6. Operation instructions

Once built, Naturalight enclosure use is straightforward. Below we describe the steps followed when the box is not integrated in the larger semi-natural living environment.

Step 1: The camera and LED plate are screwed in place on the roof with the camera, associated lens and the two chosen LEDs. A 3D-printed light trap is secured with screws around the lens.

Step #2: The living, freely moving animal are introduced in the enclosure and the roof is secured in place on top of the box using the 3D-printed brackets. The cables for the camera and LEDs are connected to power-up the components and ensure the transfer of the camera data to a PC. Additional 3D-printed light traps are set on the box handling holes. Optionally water bottles can be put in place and secured with O-rings on the outside side of the walls with the water tap protruding inside the box and accessible to the animal.

Step #3: The camera is set ON to check that animals positions and movements can be accurately tracked. The light exposure protocol with duration, wavelength and irradiance level defined in advance is applied while the animal position and movements are constantly recorded.

Step #4: After the end of the light exposure session, the LEDs are turned off, the camera and LEDs are disconnected, and the roof is removed. The rat is removed from the enclosure and returned to its housing cage.

Step #5: Any traces of urine and feces are removed, and the cage floor is gently wiped with water before introducing a new animal.

7. Characterization and validation

LEDS light spectra measurements and convolution with spectral sensitivities of photoreceptors in rodents.

LEDS light spectra have been measured using a fibered spectrophotometer (Avaspec2048, Avantes, The Netherlands). We underline that it is important to measure these spectra or obtain a device-specific spectrum from the manufacturer rather than a generic spectrum as LED spectra vary sometimes significantly from one individual component to another. Indeed, in our measurements we found a peak at 377 nm and 435 nm for the LEDs which had manufacturer specifications at 375 and 450 nm respectively. For proper estimation of light dosimetry and effective luminance (photometric parameter) at the eye level, the LEDs spectra (radiometric measurement) are convolved with the spectral sensitivity of the photoreceptors of rodents using a dedicated, freely available calculator toolbox [8]. The LED spectra and corresponding effective luminance are shown on Fig. 7 along with the corresponding photometric sensitivity for each photoreceptor.

Irradiance maps.

A power meter (PD-300-UV-SM, MKS, United Kingdom) was used to measure local irradiances in $\mu\text{W}/\text{mm}^2$ inside the enclosure at 77 regularly spaced points on a 7 by 11 grid. A cosine corrector placed on the power meter sensitive element was used to collect light within a 180° angle. The irradiance measurements grid was placed at two different heights within the normal localizations than can be reached by the animals. Fig. 8 shows the relative irradiance maps for each LED at each plane. The irradiance maps are similar for both LEDs. Overall, the inhomogeneities in irradiance are less than 25 % of the maxima. The plane at 11 cm from the floor presents less homogeneous maps especially at the side and corners, due to a shorter distance to the LEDs. In addition, there is a local decrease of irradiance close to the two apertures flanges (on the left and bottom sides) used for tunnel connection with other modules. This is due to the absence of reflective paint in the corresponding voids. In terms of absolute irradiance, it can be adjusted easily by varying the current flowing through the LED. The relationship between the irradiance and the current was found not to be linear for the LED and Led Driver used (Thorlabs, LEDD1B) thus requiring experimental measurements for calibration (see Fig. 6B). The irradiances at the center of the floor (vertically to the LED sources) were measured between 1 and $125 \mu\text{W}/\text{cm}^2$ for the range of currents (0–1.2A) delivered by the LED driver. The maximum irradiance is in the order of magnitude of what was obtained by the preliminary simulations (see Fig. 2, with a maximum of $2 \mu\text{W}/\text{mm}^2$, i.e. $200 \mu\text{W}/\text{cm}^2$ at the center of the floor).

Preliminary experiment on the effect of light on memory performance.

In rodents, moderate and even weak exposures to light have been shown to have various and either detrimental or beneficial effects on circadian rhythms, metabolism, behavior and cognition [2–3,6,11]. Recent studies have shown that blue light, via melanopsin receptor ganglion cells in the retina, is particularly involved in the effects of blue light exposure [4,8]. In a first preliminary study, the Naturalight module was used as a standalone module to study impact of light exposure on rat cognitive performance.

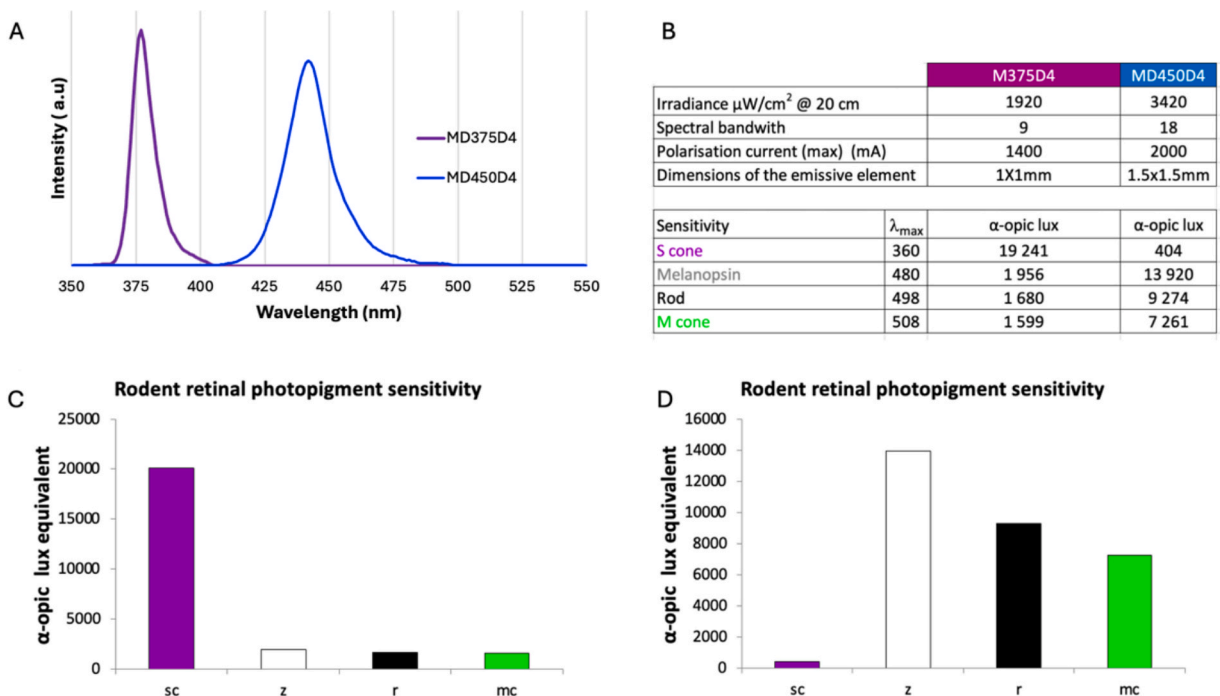


Fig. 7. A) Spectra of the LEDs used in the study. B) Radiometric and photometric characteristics of the LEDs. C) Photometric characteristics of the LEDM375D4 for the rodent photoreceptor D) Photometric characteristics of the LEDM450D4 for the rodent photoreceptor. sc = S-cones, Z = melanopsin receptors, r = Rods, mc = M-cones.

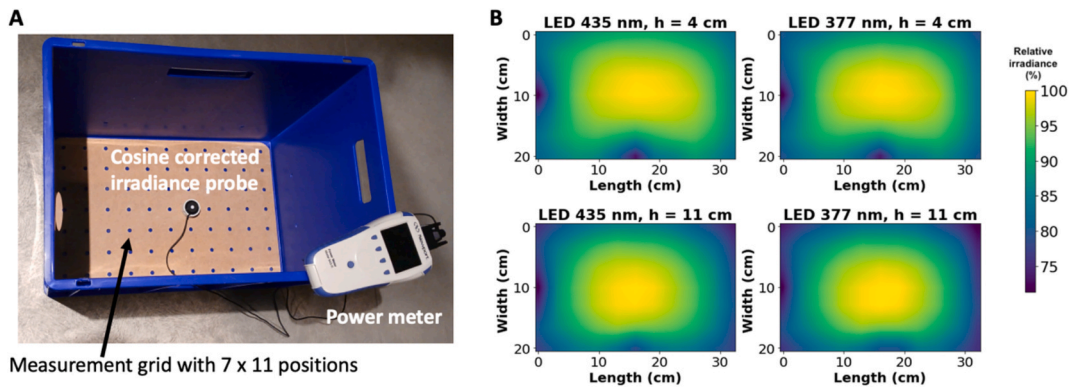


Fig. 8. A) Measurement set-up showing the 7x11 positions grid at the bottom of the enclosure with the power meter probe at one position. B) Relative irradiance measurement at two heights relative to the floor for two LEDs MD375D4 (experimental peak at 377 nm) and MD450D4 (experimental peak at 435 nm).

8. Material and methods

8.1. Animals

The experiments were carried out according to the ethical guidelines of the European Communities Council Directive 2010/63/EU, as well as the approval of the “Structures in charge of Animal Welfare” (SBEA) of the “Lyon Neuroscience Research Center”. Care was taken at all stages to minimize stress and discomfort to the animals. Data were obtained from 10 female Long Evans rats (Charles River, L’Arbresle, France). At their arrival, rats were introduced all together in the naturalistic environment. They were maintained under a 12/12 h light/dark cycle (lights on from 7 A.M. to 7P.M.). Food and water were available ad libitum in the living environment.

8.2. Daily light exposure

The group was divided into two subgroups exposed to light sources of different wavelengths. We chose two wavelengths: one (violet light, 377 nm) activating only violet light receptor cones, the other (blue light, 435 nm) activating mainly melanopsin receptors (see Fig. 7 C and D). The irradiance magnitude was set to low levels of about $5 \mu\text{W}/\text{cm}^2$ for both wavelengths. Light exposure was carried out in the Naturalight module used as a standalone module outside the living environment in order to have the same total duration of illumination for all animals in this preliminary experiment. Rats were exposed for one hour a day for twenty days, between 12p.m. and 5p.m. This period was chosen because it corresponds to a period of sleep for the rats, based on the observation of their activity on 48-hours videos. Water was available ad libitum during light exposure.

8.3. In-place and in-context object recognition test

After twenty days of light exposure, a test involving both spatial memory and object recognition in context was carried out to observe any potential impact of light on cognitive performance [12–13]. Novel object recognition task is based on the natural tendency for a rat to explore more a novel object than a known one. Thus, rats decrease their exploration of an object when encountered for the second time and this decrease could be interpreted as the recognition of the object [14]. In the task used in this preliminary experiment, objects were also associated to a specific location in a specific context (Fig. 9). In practice, rats were first accustomed to the empty test arena. This arena was a 57 x 52 x 40 cm cage placed in a sound proofing box illuminated with white light. There was no water available in test arena. The task itself (see protocol on Fig. 9) began with a first encoding phase, during which two different objects were

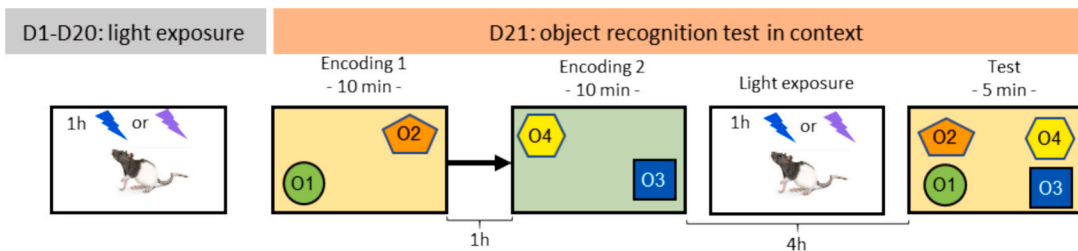


Fig. 9. In-place and in-context object recognition test protocol. O1, O2, O3, O4 correspond to the different objects used in the test. Yellow and green backgrounds correspond to the first and second context respectively.

presented in a given context and in opposite corners of the cage. One hour later, a second encoding phase, including two other different objects presented in a different context and in different places, was carried out. The two contexts are defined by the floor of the experimental cage. These two removable floors exhibited marked differences in both texture and color. The test itself took place after a four-hour delay, during which the animals were exposed in the Naturalight module for one hour to light (LED with peak at 377 nm or 435 nm depending on the group). In this test, the same four objects as the one used in the encoding phases were presented in the first context, some of them in different places (Fig. 9). During the whole experiment, animal behavior was monitored and recorded via the camera placed above the experimental arena. V ideo data were used to produce trajectories of animals with the machine learning software “DeepLabCut” (LGPL license, <https://www.mackenziemathislab.org/deeplabcut-home>). Based on these trajectories, exploration time of each object was measured. The exploration of an object was defined by the presence of the rat snout at a distance less than 2 cm from the object. Given the small numbers of animal in each group, non-parametric statistical tests were used. The significance threshold was set at $p = 0.05$.

9. Results and discussion

The principle of this test is based on the natural ability of rodents to explore more what seems new to them.

Therefore, if the animal has formed a memory of an object at a given place in a given context, this object will be recognized and less explored. In the case of our test, if the rat has integrated the elements of each set proposed during encoding, we can make the following predictions (see Fig. 9):

- Object #1 is an object seen in this same place and in this same context as during the encoding phase Therefore, it should be the least explored.
- Object #2 was seen in this context during encoding but has been moved. It should be more explored than object #1
- Object #3 is in its original place but in a different context. It should also be more explored than object #1
- Object #4, is in a different context and has been moved and should be the most explored of the four objects

An analysis of the exploration times for each object during the encoding phases did not reveal any difference between the two experimental groups. All the animals explored the two proposed objects equally during each encoding phase whatever the illumination wavelength.

The results of the test phase are shown in Fig. 10. Only the group of animals exposed to blue light showed results in line with the prediction, with a tendency ($p = 0.075$) for object 4 to be more explored after illumination with blue light compared to violet light and object 1 little explored. In contrast, animals exposed to violet light showed no preference. Although caution is required given the small number of animals in each group in this preliminary experiment, animals exposed to blue light show performance in line with predictions whereas memory for objects in place and in context is completely impaired in those exposed to violet light.

In our protocol, both groups were subjected to two types of light exposure: a chronic exposure for 20 days and a one-off exposure during the retention period. Chronic exposure does not seem to induce any difference between the two groups, which behave in the same way at the time of encoding. We can then hypothesize that it was mainly the difference in light during the retention period that affected memory performance. These preliminary results deserve further experiments but are, however, in line with previous work showing that light exposure could therefore have a stimulating, awakening effect, depending on the time of day the subject is exposed to it [6,15].

9.1. Capabilities and limitations of the hardware

- Homogeneous illumination of rodents is obtained within a living module compatible with behavioral experiments. The light exposure was found to be homogeneous (less than 25 % change between the center and sides of the enclosure)
- The illumination was characterized photometrically by convolving the measured LEDs spectra with rodent photoreceptor sensitivity using a dedicated, freely accessible toolbox [8].
- LEDs at two wavelengths (377 nm and 435 nm) were used, but any wavelength can be easily added and the corresponding illumination characterized both radiometrically (irradiance) and photometrically (convolution with photoreceptors spectral sensitivity)
- The irradiance level of the exposure can be adjusted between several $1 \mu\text{W}/\text{cm}^2$ up to $125 \mu\text{W}/\text{cm}^2$ at the level of Naturalight enclosure floor.
- LED can be remotely set ON (at a preset irradiance value) or OFF using a TTL signal as a trigger.
- The irradiance can be modulated either manually or using the 0–5 V modulation signal to tune the LED current.
- Automated patterns of light exposure could be easily designed with controlled timing and exposure level using modulation signal.
- As the roof is relatively heavy (a few kg), simple improvements of the design would be to (i) to decrease the wall by using lighter X-rails and walls materials (ii) add a hinge on one of the largest sides of the roof to make its handling easier.
- The wall painting degrades with time due to animal scratching. A foreseen improvement is to use a matte varnish to protect the paint while maintaining its optical diffusion properties.
- Animals' behavior and trajectories are monitored and recorded automatically using cameras, more accurate estimation of the light exposure inside the Naturalight module of each animal could be obtained using recorded trajectories to weight the specific light exposure according to the experimental irradiance maps.

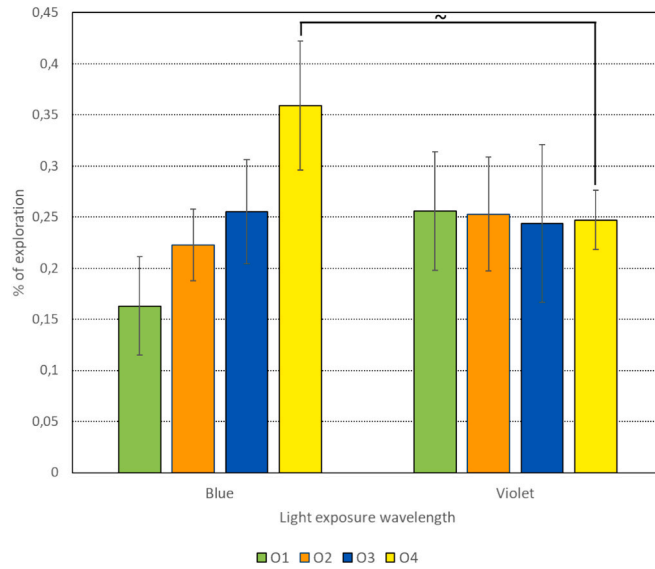


Fig. 10. Proportion of time spent exploring each object during the test phase relative to the total exploration time. ~: $p = 0.075$ (Kruskal-Wallis test).

- In future experiments, the Naturalight module could be integrated into the semi-natural living environment as already tested (Fig. 11). Animals implanted with RFID tags can be identified and tracked inside the Naturalight module, in order to estimate their individual exposure time.

CRedit authorship contribution statement

Christophe Hecquet: Writing – review & editing, Methodology, Investigation, Data curation. **Maylis Etcheberry:** Investigation, Formal analysis. **Nadine Ravel:** Writing – original draft, Supervision, Methodology, Investigation, Conceptualization. **Philippe Litaudon:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Conceptualization. **Frédéric Pain:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

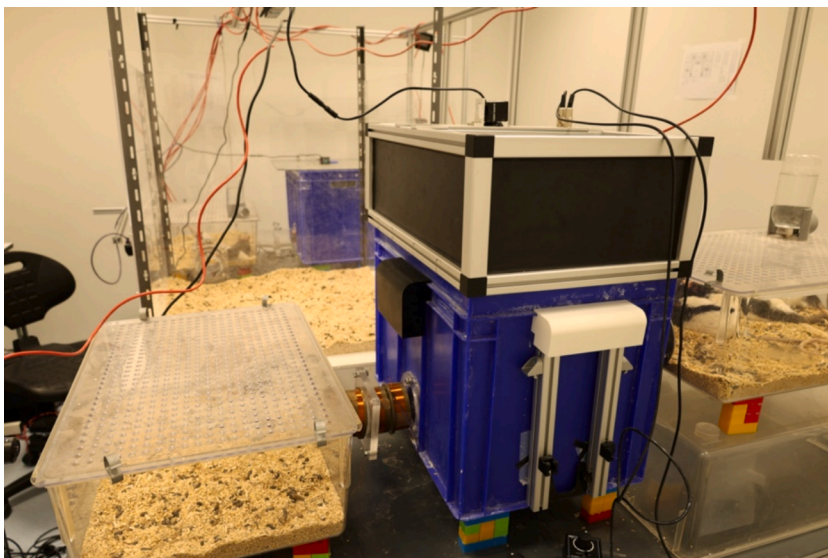


Fig. 11. Naturalight enclosure integrated in a multimodule living environment for freely behaving rats.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Authors would like to thank Noam Atlas and Jean René Rullier from LCF for help with the mechanical parts and Marc Apfel also from LCF for help with electronics.

Ethics statements

The experiments complied with the ARRIVE guidelines and were carried out according to the ethical guidelines of the European Communities Council Directive 2010/63/EU, as well as the approval of the “Structures in charge of Animal Welfare” (SBEA) of the “Lyon Neuroscience Research Center”.

Funding

This work was supported by CNRS Mission pour les Initiatives Transverses et Interdisciplinaires « Vie et Lumière 2022-2023 Program ». M Etcheberry's internship was funded by Université Lyon 1.

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