A low-cost device for cryoanesthesia of neonate rodents

Authors

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Abstract

Studying the development of neural circuits in rodent models requires surgical access to the neonatal brain. Since commercially available stereotaxic and anesthetic equipment is designed for use in adults, reliable targeting of brain structures in such young animals can be challenging. Hypothermic cooling (cryoanesthesia) has been used as a preferred anesthesia approach in neonates. This commonly involves submerging neonates in ice, an approach that is poorly controllable and potentially stressful to animals. We have developed an affordable, simple to construct device – CryoPup – that allows for fast and robust cryoanesthesia of rodent pups. CryoPup consists of a microcontroller controlling a Peltier element and a heat exchanger. It is capable of both cooling and heating, thereby also functioning as a heating pad during recovery. Importantly, it has been designed for size compatibility with common stereotaxic frames. This open-source device will facilitate future studies into the development of neural circuits in the postnatal brain.

Keywords

Cryoanesthesia; Rodents; Neonates; Peltier; Stereotaxic surgery; Thermoelectric device

Specifications table

Hardware name	CryoPup
Subject area	Neuroscience
Hardware type	Hypothermic anesthetic device
Open source license	GNU General Public License 3.0 (GPL 3.0)
Cost of hardware	Materials (161 GBP) + PCB (45 GBP) + CNC (96 GBP) = 302 GBP per unit (without shipping costs)
Source file repository	https://osf.io/mxgz8/ https://github.com/FrancisCrickInstitute/Cryopup

1. Hardware in context

Systems neuroscience research heavily relies on visualizing and manipulating defined neural circuits in the brain. In rodent models, this is commonly achieved via delivery of viral vectors to the brain using stereotaxic surgery [1,2]. For this purpose, animals are secured into a stereotaxic frame while anesthetics are delivered via intraperitoneal injection or inhalation through a mouthpiece [3].

While this procedure is routinely used in adults, it is suboptimal for neonates, which are increasingly used to study how neural circuits develop postnatally [4–8]: inhalation anesthetics can negatively and lastingly affect the brain of neonate rodents, in particular areas associated with memory [9–12]. Instead, cryoanesthesia, i.e. anesthesia by deep hypothermia, has been commonly used in such young animals. This is typically carried out by submerging neonates in crushed ice or using simple cooling pads [7,8,13–15]. These approaches, however, do not provide accurate or consistent temperature control. A system capable of adjustable temperature control for hypothermic surgeries in adult rodents was recently published, but it is incompatible with stereotaxic surgeries due to its dimensions [16].

We have designed a device (CryoPup) to overcome these limitations. Based on a bidirectionally controlled Peltier element, CryoPup is capable of rapid cooling and heating (temperature range 0ºC to +42ºC), thus also acting as a heating pad. Due to its compact size, it is fully compatible with modern stereotaxic equipment. We have also designed a 3D printed head holder which facilitates the positioning of neonates during stereotaxic surgeries, thus increasing targeting success. Finally, we show that CryoPup allows for rapid, reliable and safe cryoanesthesia in mouse neonates.

This device thus represents a cost-effective and versatile solution for cryoanesthesia in neonate rodents.

2. Hardware description

CryoPup combines a thermoelectric module (Peltier element) which rapidly cools to temperatures suitable for cryoanesthesia of rodent neonates, with 3D printed components to form a surgery bed. Peltier-based cooling provides accurate temperature control for cryoanesthesia. Temperature is set via an intuitive control panel and the device is compatible with standard stereotaxic frames without further modifications. A 3D printed head holder ensures cranial stability during stereotaxic surgeries. Importantly, CryoPup can seamlessly switch from cooling to heating, and thus acts as a heating pad during recovery, or during the use of other forms of anesthesia in adults. This device is also equipped with a temperature probe that can be used to monitor external or internal (e.g. rectal) temperature.

Main components

The device is composed of a main printed circuit board (PCB), the heat removing system and the bed which encloses a thermoelectric element (Fig. 1a-b).

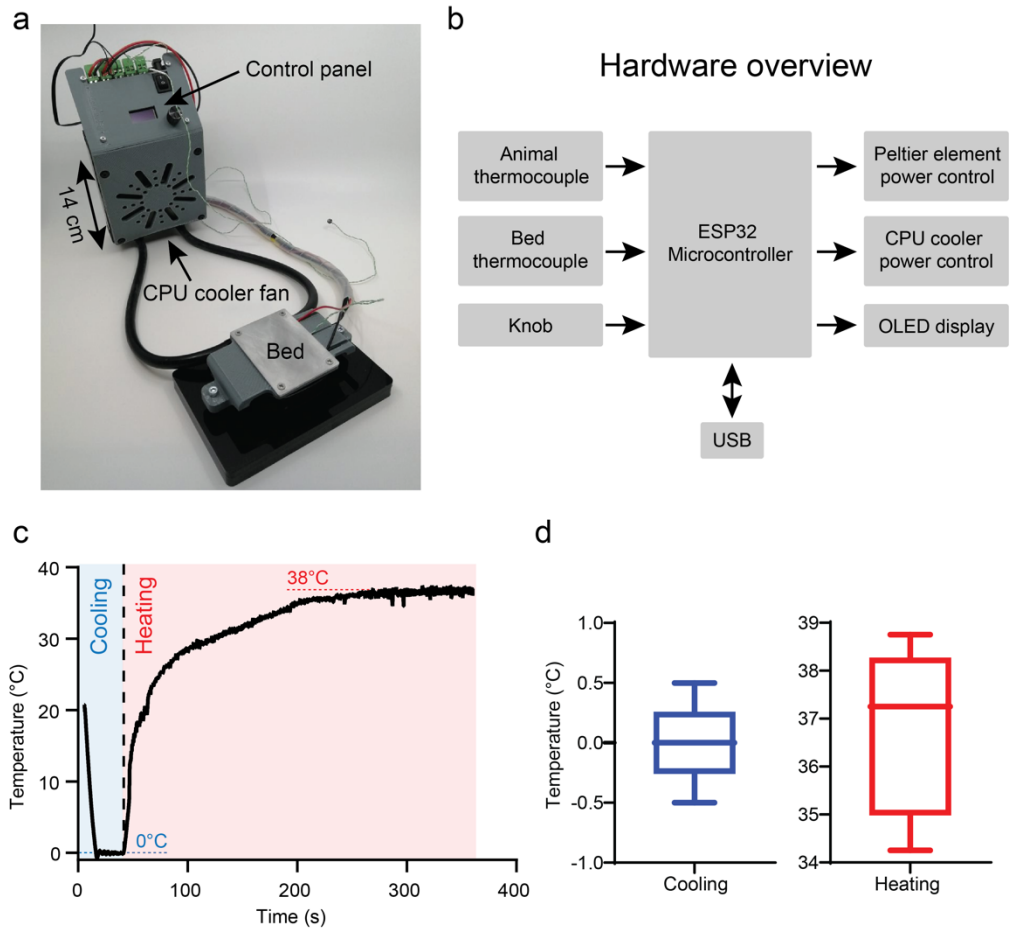

Fig. 1. CryoPup device (a) and schematic control diagram (b). Device performance in a cooling (target temp. 0°C) and heating (target temp. 38°C) cycle (c). Temperature variation around the setpoints (d). Box-and-whisker plots show median, interquartile range and range.

Electronics

The PCB hosts a microcontroller (Espressif ESP32) which controls the Peltier element and heat exchanger (CPU water cooling loop). Two Maxim integrated thermocouple amplifiers and digital converters (MAX31855KASA+T) acquire data from two Type-K temperature sensors: one in the bed and one external sensor which can be used to monitor experimental animals' temperature. Two power MOSFETs (IRFH8318TRPBF) control the power to the All-In-One liquid cooler (Corsair Hydro H60) and the thermoelectric element (TEC1-12709). A rotary encoder is used to set the desired bed temperature. The desired temperature as well as the two thermocouple temperatures are continuously displayed on a 0.96-inch OLED display.

Firmware

CryoPup is capable of both cooling and heating. Rapid cooling is achieved by the thermoelectric element pumping heat over a short distance and by the highly effective heat removal by the closed loop liquid cooler (Fig. 1c). Bed temperature is adjusted combining two bang-bang control algorithms. When cooling (i.e. desired bed temperature is below a predefined ambient

temperature) the microcontroller modulates power to the Peltier element using a bang-bang control while the heat removing system is constantly ON. In contrast, if a higher-than-ambient desired temperature is selected, the heat removal system shuts down and a bang-bang control with a small pulse width modulation (PWM) duty-cycle value is initiated. With the thermoelectric element operating in its inefficient regime, heat gradually accumulates on the bed. The oscillations (ringing) around the set temperature are bigger when warming (± 1.5°C) than cooling (± 0.5°C) – thus precise temperature control is ensured during cryoanesthesia (Fig. 1d).

3. Design files

The design files provide all necessary content to manufacture, assemble and use CryoPup.

Table 1. List of design files

Design filename	File type	Open source license	Location of the file
Control board	Easy EDA/ Gerber/csv	GPL-3.0 License	OSF
Peltier holder	Autodesk Inventor/STL/Step	GPL-3.0 License	OSF
Bed plate	Autodesk Inventor/STL/Step	GPL-3.0 License	OSF
Electronics holder	Autodesk Inventor/STL/Step	GPL-3.0 License	OSF
Base	Autodesk Inventor/DXF/STL/Step	GPL-3.0 License	OSF
Base spacer	Autodesk Inventor/DXF/STL/Step	GPL-3.0 License	OSF
Head holder	STL/Step	GPL-3.0 License	OSF

Control board: Design and manufacturing files for the PCB as well as Pick and Place (PnP) and Bill of Materials (BOM) files for turn-key PCB manufacturing and assembly.

Peltier holder: Design and manufacturing files for 3D printing the bed frame.

Bed plate: Design and manufacturing files needed for machining the aluminium bed plate.

Electronics holder: Design and manufacturing files for 3D printing the control panel frame which is attached to the heat exchanger and holds the PCB.

Base: Design and manufacturing files for 3D printing or laser cutting the bed base.

Base spacer: Design and manufacturing files for 3D printing or laser cutting the base spacer for the bed.

Head holder: Design and manufacturing files for 3D printing the neonate stereotaxic head holder.

4. Bill of materials

The components required to build CryoPup, and their costs, are listed in Table 2.

Table 2. Components required for CryoPup

Designator	Component	Number	Cost per unit (£)	Total cost (£)	Source of materials	Material type
Peltier element	TOOGOO(R) 100W TEC1-12709 Thermoelectric Cooler Peltier 12V	1	7.39	7.39	Amazon	Semiconductor / Ceramic
CPU liquid cooler	Corsair Hydro H60 Liquid CPU Cooler	1	65.99	65.99	Amazon	Copper / Aluminium / Polymers / Water-Glycol
OLED display	0.96-inch OLED Display SSD1306 128x64 Pixels	1	4.99	4.99	Amazon	Glass / Semiconductor
ON/OFF switch	RS PRO SPST, Off-On Rocker Switch Panel Mount	1	1.36	1.36	RS Components	Polymer/ Copper
Type K Thermocouple (Bed)	Type K Thermocouple 1m Length, 6.35mm Diameter +200°C	1	11.93	11.93	RS Components	Copper / Metal Alloys
Type K Thermocouple (Animal)	RS PRO Type K Thermocouple 1m Length, 2mm Diameter +250°C	1	3.59	3.59	RS Components	Copper / Metal Alloys
Compression Spring	Hilka 79552020 Spring Assortment, 200 Piece	2	6.00	6.00	Amazon	Steel
Thermal compound	Metal Oxide Thermal Paste, 0.65W/m·K	1	6.24	6.24	RS Components	Silicone / Zinc Oxide
Power supply 12V 90W	RS PRO 12V DC Power Supply, 90W, 7.5A, C14 Connector	1	51.23	51.23	RS Components	Polymers / Semiconductors / Metals
M3 screw x 4mm	RS PRO, Pozidriv Pan Steel, Machine Screw DIN 7985, M3x4mm	2	0.025	0.050	RS Components	Steel

M3 screw x 10 mm countersunk	RS PRO, Pozidriv Countersunk A4 316 Stainless Steel, Machine Screw DIN 965, M3x10mm	4	0.056	0.224	RS Components	Steel
M3 screw x 16 mm	RS PRO, Pozidriv Pan Steel, Machine Screw DIN 7985, M3x16mm	4	0.036	0.144	RS Components	Steel
M4 screw x 30 mm	RS PRO Plain Stainless-Steel Hex Socket Cap Screw, DIN 912 M4 x 30mm	2	0.6	1.2	RS Components	Steel
M3 nut	RS PRO Stainless Steel, Hex Nut, M3	8	0.03	0.24	RS Components	Steel
M3 threaded insert	RS PRO, M3 Brass Threaded Insert diameter 4mm Depth 4.78mm	2	0.13	0.26	RS Components	Steel
M4 threaded insert	RS PRO, M4 Brass Threaded Insert diameter 5.6mm Depth 7.95mm	2	0.14	0.28	RS Components	Steel

5. Build instructions

This section describes the build instructions for the PCB, bed base and electronics holder.

5.1 Required tools

1. FDM 3D printer (a Zortrax M200 with 200 × 200 × 180 mm building volume was used here)
2. Phillips screwdriver
3. Hex key set
4. Pliers
5. Side cutters / Cable strippers
6. Soldering iron and solder

5.2. Manufacturing the custom-designed PCB

The following manufacturing and assembly files for the PCB are available on the OSF repository (link): Gerber files which contain the computer-aided manufacturing files, the bill of materials (BOM) that contains the part number associated with each component and the pick and place file (PnP) which specifies the coordinates on where each component must be placed by the pick and place machine. Following the simple steps outlined below, the board can be reproduced by the manufacturer (JLCPCB) without the need for ordering or soldering components.

1. Download the files from the OSF repository: link.
2. Create customer account on JLCPCB and sign in.
3. Go to JLCPCB instant quote and add Gerber file.
4. Select default settings and preferred PCB color.
5. Under PCB settings, select SMT assembly and indicate quantity (2 or 5).
6. Click next and add the BOM and PnP files.
7. Click next and review if any parts are unavailable. If yes, order from a different supplier (e.g. Mouser, Farnell, Digikey …).
8. Click next to see the position of each part on the PCB; save to cart and complete payment.

A more detailed document describing the procedure with images can be found on the repository manufacturing files folder (link).

5.3. Procuring the remaining components

A comprehensive Bill of Materials can be found in section 4.

5.4. Manufacturing of bed base and electronics holder

The four components to be 3D printed can be found in the manufacturing files/mechanical parts folder. The base and the base spacer parts can also be 3D printed but were laser cut from 10 mm acrylic sheets here. M4 and M3 threaded inserts are installed in the base and the Peltier holder parts (Fig. 2a).

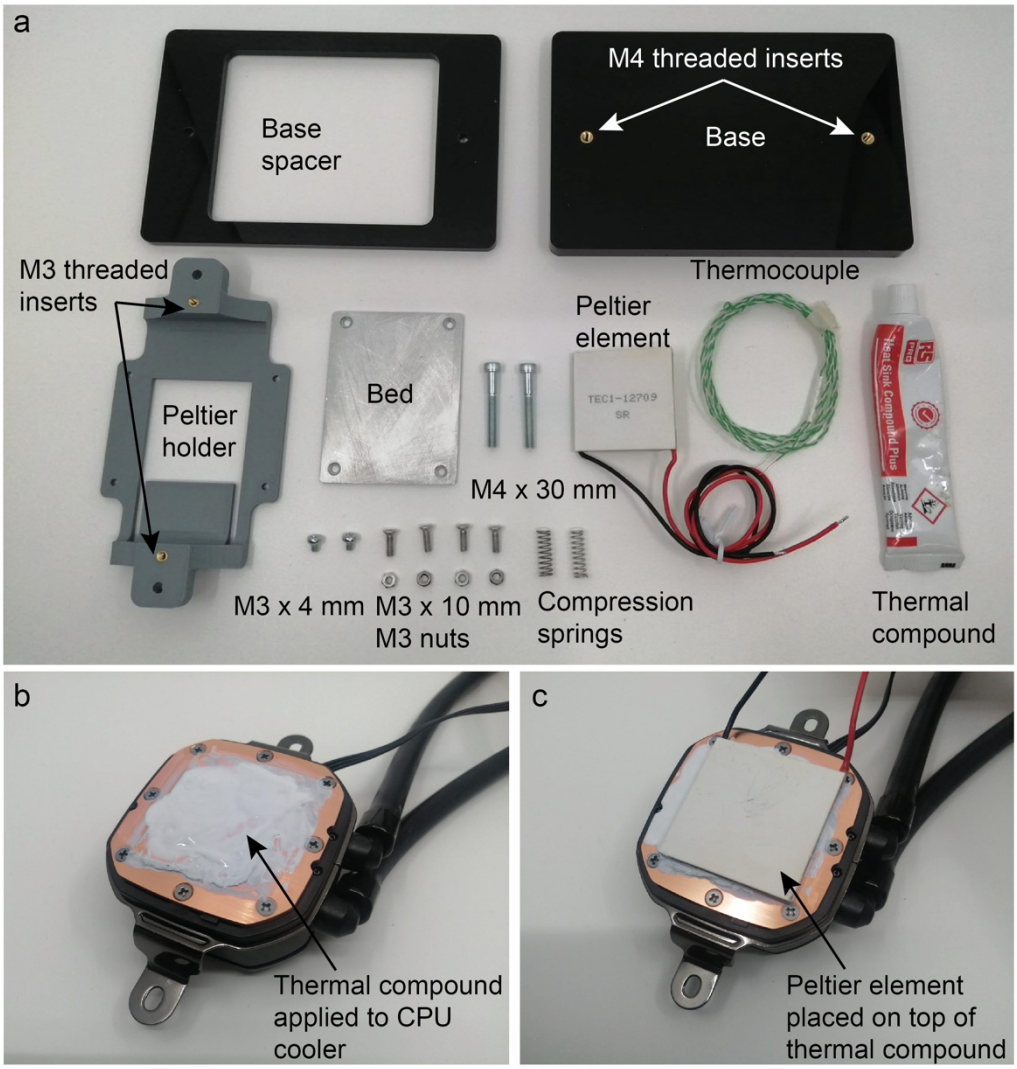

Fig. 2. Required parts for the bed build (a). Application of thermal compound to top of cooler block (b). Peltier element placed on top of thermal compound with cold side facing up (location of red and black wires used as reference) (c).

5.5. Manufacturing the bed

The bed is manufactured from aluminium to ensure good temperature surface homogeneity. This was outsourced to a third-party manufacturer (Hubs), using standard settings (aluminium 6082, no additional treatments). The step files in the Manufacturing files/Mechanical parts folder can be directly uploaded to the instant quote webpage.

5.6. Bed assembly

After manufacturing all bed components (Fig. 2a), thermal compound is applied to the CPU cooler surface (Fig. 2b). The thermoelectric element is installed on top of the heat sink compound with the writing (hot side) facing down (see Fig. 2c on how to check polarity).

The 3D printed Peltier holder is placed around the Peltier element (Fig. 3a) and attached to the CPU cooler using two 4 mm long M3 screws (Fig. 3b). The bed thermocouple is inserted into groove of the bed aluminium plate (Fig. 3c), the bed plate placed on top of the Peltier element enclosing the thermocouple and secured with 10 mm long countersunk M3 screws and nuts (Fig. 3d-e). The bed is attached to the base and base spacer using two M4 screws with a compression spring between the base and the Peltier holder (Fig. 3f-h). These springs allow for easy adjustment of bed height.

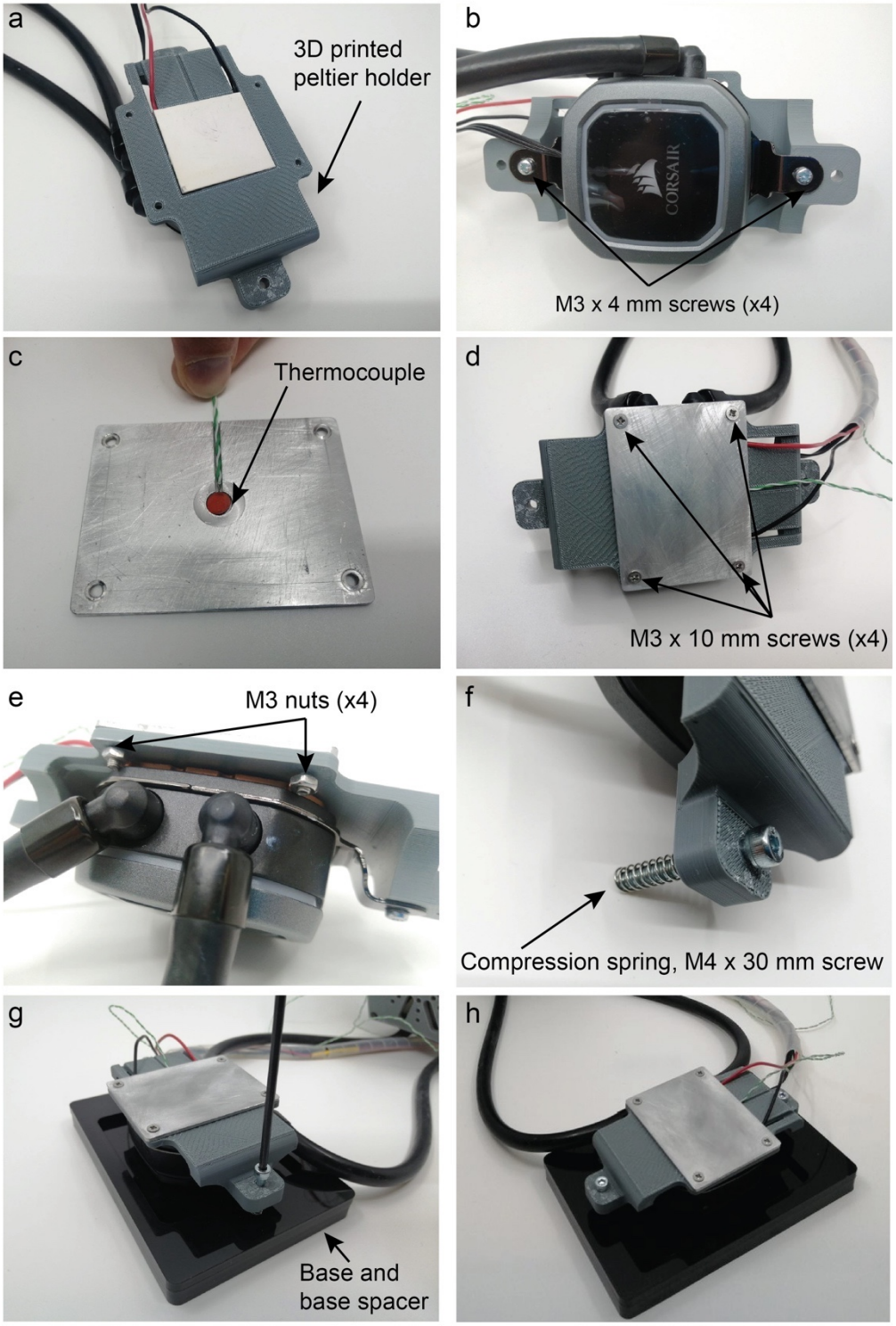

Fig. 3. Installation of the thermoelectric module in the holder (a). Peltier holder attached to CPU cooling block (b). Thermocouple placed between Peltier element and bed aluminium plate (c). Aluminium plate attached to Peltier holder (d). Bed plate and Peltier holder (e). Compression springs provide counterforce to adjust height and to level the bed (f). Bed attached to laser cut base and base spacer (g). Finished assembly (h).

5.7. Electronics holder assembly

The OLED display and the ON/OFF switch are the final components to be soldered on the board. It is recommended to apply an isolating layer (electric or polyimide tape) to the back of the display before soldering it on the board (Fig. 4a).

The firmware must be uploaded to the board before assembling it in the 3D printed electronics holder since the micro-USB port cannot be accessed afterwards. To upload the firmware on the board, follow the next steps (Windows):

1. Install the Arduino IDE on your computer (link)
2. If necessary, install the SiLabs CP2104 drivers (link)
3. Install the ESP32 package for the Arduino IDE (link)
4. Download the sketch from the repository (link)
5. Download libraries:
    i. ESP32_analog_write (link)
    ii. Encoder.h (link)
    iii. Adafruit_MAX31855.h (link)
    iv. Adafruit_GFX.h (link)
    v. Adafruit_SSD1306.h (link)
6. Connect the CryoPup board to the computer using a micro-USB cable and check “Device Manager” for the name of the COM port.
7. Select the board on Tools -> boards -> ESP32 Arduino -> Adafruit ESP32 Feather
8. Select the board on Tools -> ports -> the COM port listed in Device Manager.
9. Open the sketch *Cold_bed_2.ino* and upload it to the board (link)

After uploading the firmware, the PCB is attached to the 3D printed electronics holder using four 16 mm long M3 screws and nuts (Fig. 4b). The original fan screws are subsequently used to attach the electronics holder to the fan shroud (Fig. 4c). The frame also serves as a finger (and cable) guard. Finally, all electrical connections (for two thermocouples, CPU fan and pump, and Peltier element) are completed as specified in Fig. 4d. Since the CPU closed loop water cooler consists of two main elements (heat exchanger, i.e. fan and radiator, and pump/cooling block inside the bed) which have different wiring connectors, they must be re-wired and connected to the same screw terminal as outlined in Fig. 4e.

5.8. Rectal probe assembly (optional)

The external thermocouple can be used as a rectal probe to monitor animal temperature. For this purpose, the end of the external thermocouple probe is covered with a 2cm piece of Tygon® E-3603. This device is implantation biocompatible (USP class VI).

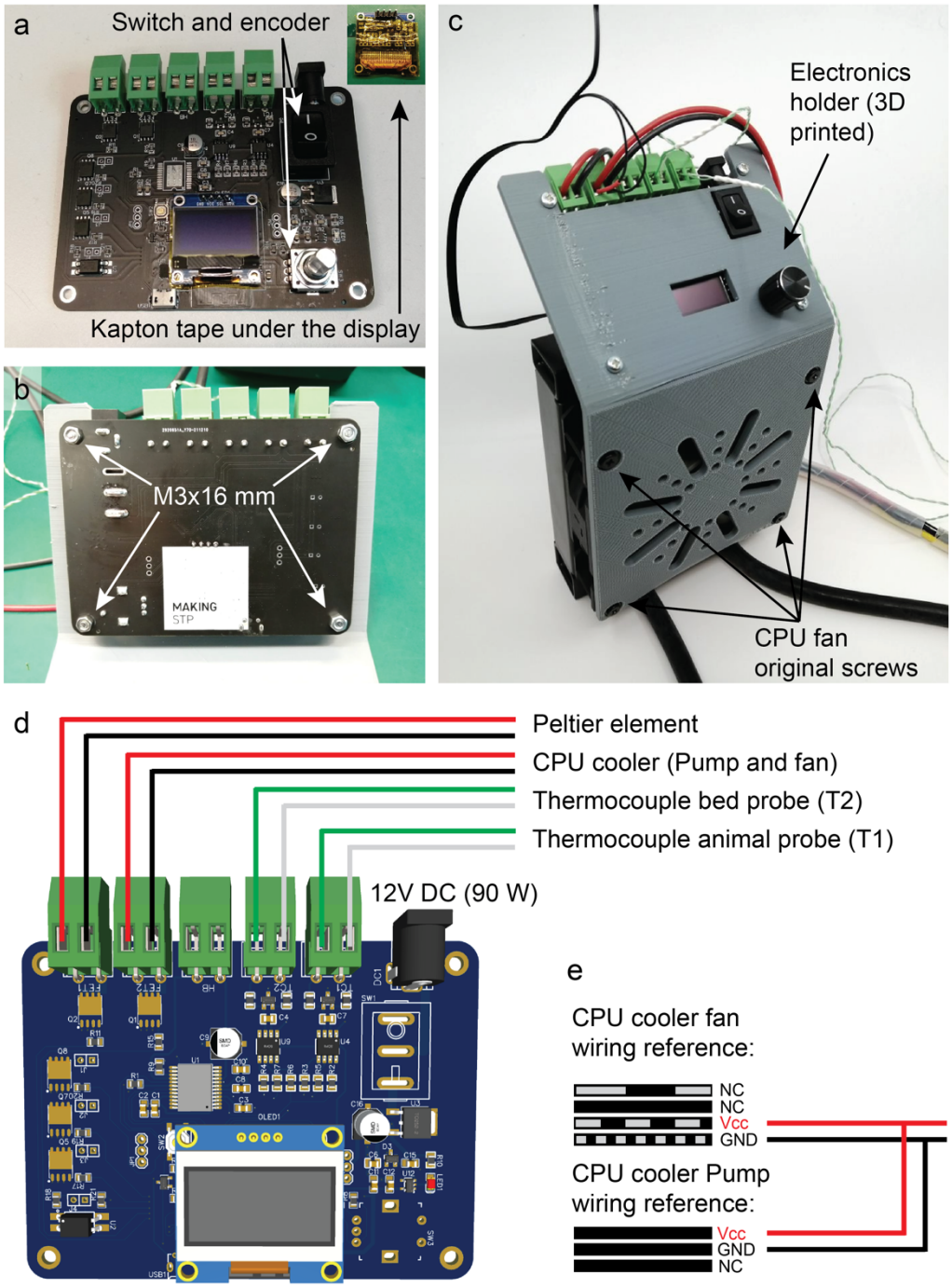

Fig. 4. PCB with components and insulation between back of screen and microcontroller housing. PCB is fixed to the 3D printed electronics holder (b). Electronics holder attached to heat exchanger using the original fan screws (c). Electrical connections to/from PCB screw terminals (d). Heat exchanger fan and pump wire connections (e).

6. Operation instructions

This section describes detailed steps for operating CryoPup.

1. Connect the 12 V power supply to the barrel jack (Fig. 5a).
2. Power up using the ON/OFF switch (Fig. 5b).
3. The display will show three parameters: the temperature from the external thermocouple (T1), the temperature from the bed (T2) and the desired temperature of the bed (DT). DT can be selected by the user at any time using the selection knob (default = 0°C).

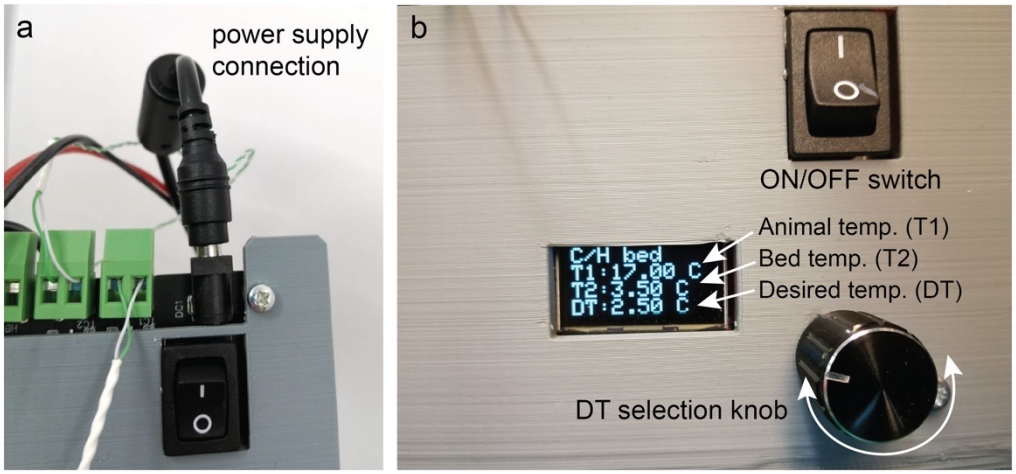

Fig. 5. CryoPup is powered from a 12V DC 90W center positive power supply via a standard 2mm DC barrel jack connector (a). CryoPup control panel (b).

7. Validation and characterization

To test the functionality of CryoPup, we used it to cryoanesthetise mouse neonates (postnatal day 2-3, n = 9) (Fig. 6a). Briefly, CryoPup was set to a desired temperature (DT) of 2ºC to ensure a bed temperature (T2) of 4°C. Mouse pups were gently placed onto the device bed with surgical tape to ensure that the bed was in close contact with the pups' chest/abdomen (Fig. 6b). The pedal withdrawal reflex was tested every 30s with blunt forceps on alternating feet, and mice were assessed to have a level of anesthesia appropriate for surgery (surgical plane) when this reflex was absent from both feet. This was achieved in 123.8 ± 8.3 s (mean ± SEM; Fig. 6c, e).

Following induction, pups were maintained at 4°C for 15 minutes before DT was set to room temperature (21°C). The pedal withdrawal reflex was again monitored, and recovery was considered complete either when this reflex returned or when mice spontaneously began to move away from the metal bed (140.2 ± 7.6 s, mean ± SEM; Fig. 6d, e). Mice were then moved to a recovery chamber and then returned to the maternal nest.

CryoPup is thus capable of (1) rapidly inducing cryoanesthesia, (2) stably maintaining this state for periods of time compatible with surgical procedures such as intracranial stereotaxic injections, and (3) safely returning experimental subjects to wakefulness.

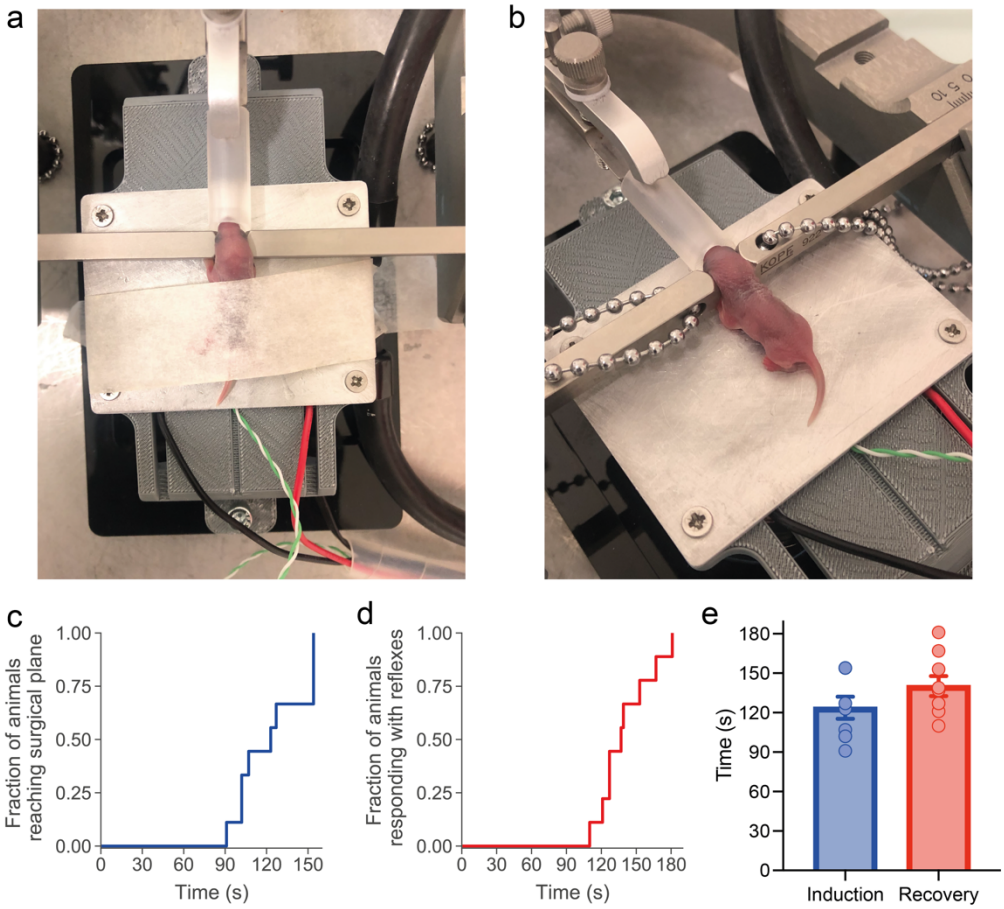

Fig. 6. Example images of mouse neonates during cryoanesthesia on CryoPup (a, b). Survival curves showing the time of induction to reach surgical plane (c), and recovery rate following cessation of cryoanesthesia (d). Average induction and recovery latencies (e). Data presented as mean ± SEM.

8. Conclusion

We describe the design for the hardware and software of the CryoPup hypothermic anesthesia device. We provide manufacturing instructions for CryoPup and validate its use as an anesthetic device for rodent neonates. CryoPup will enable researchers to carry out controlled hypothermic anesthesia on neonate rodents and can seamlessly integrate into existing stereotaxic frames. This device thus provides a simple and cost-effective manner for stereotaxic surgery in rodent neonates, facilitating the study of developing brain circuits.

Declaration of Competing Interest

None.

Acknowledgements

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Ethics statements

Mice were bred and maintained at the specific pathogen-free mouse facility of Paris Brain Institute, with controlled temperature (21 ± 1°C) and humidity (40-70%), *ad libitum* access to standard mouse chow and water, and on a 12h:12h light/dark cycle (lights on at 8 am). Lighting cycles with a controlled rising and decreasing gradient at 8 am/8 pm respectively, simulating natural light. All procedures followed the European legislation for animal experimentation (directive 2010/63/EU).

CRediT author statement

BBJ: Conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing - review & editing, visualization; **XC-F:** conceptualization, methodology, mechanical design, electronics design, software, data curation, writing – original draft, writing - review & editing, visualization; **GK:** conceptualization, methodology, software, data curation, writing – original draft, writing - review & editing, visualization; **EdL:** Resources; **NR:** Resources, project administration; **AI:** writing - review & editing, project administration, supervision; **JK:** Conceptualization, methodology, resources, writing – original draft, writing - review & editing, project administration, supervision.

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