Variation in the onset of parental behaviours across four species of *Peromyscus* mice with different mating systems

SUPPLEMENTARY MATERIAL

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SUPPLEMENTARY FILE LIST

- Attraction-aversion_test.py - Python script used for the test of attraction/aversion to a pup.
- Pmaniculatus_normal-mating.h264 - Exemplary video of a mating event in *P. maniculatus bairdii* with all components of a typical mating: chasing, mounting, thrusting and post-coital licking of genitals.
- CameraHolder_MouseCage.dxf and CameraHolder_RatCage.dxf - Design of the camera holder made in Autodesk Inventor Professional 2016 (http://www.autodesk.com), attachable to a standard mouse cage card holder.
- Mating_monitoring.py - Python script used for the monitoring of breeding cages.
- Attraction-aversion_duration.xlsx - Raw data used for the decision about the duration of the test of attraction/aversion to a pup.
- Preliminary_data.xlsx - Preliminary parental behaviour data for four mice

MATING MONITORING WITH THE RASPBERRY PI ZERO

Here is a tutorial that describes how to construct a home-made set up to record up to 9 hours of video of a standard Allentown mouse or rat cage (Allentown, Allentown, NJ, USA) in the dark.

Material

If applicable, follow the provided link to find the website where the item can be purchased from:

1. For the Raspberry Pi
    - Raspberry Pi Zero version 1.3
    - MicroUSB charger (e.g. smartphone charger)
    - Mini HDMI to HDMI/VGA/DVI cable
    - USB OTG cable
    - Pi Zero protector
    - MicroSD card 16 or 32GB
    - MicroSD card reader
    - Yoobao YB-M4 battery pack
    - 2x20 Male header strip
    - USB mini hub
    - IR LED strips (3 LEDs per Pi Zero)
2. For the camera
    - Raspberry Pi Zero v1.3 Camera Cable
    - Pi NoIR camera module
    - 18-8 stainless steel hex nuts (thread size: 2-56)
    - 18-8 stainless steel socket head Screws (thread size: 4-40, thread length: 1 3/4")
    - Stainless steel socket head screws (thread size: 2-56, thread length does not matter).

- Fisheye lens
- 1/4"-thick cast acrylic

3. For the Real Time Clock
    - DS1307 Real Time Clock Assembled breakout board and battery
    - 1x20 or 2x20 GPIO female header
    - Wifi USB dongle

Procedure

Configuring the SD card

Download the zip file containing the operating system called NOOBS from the Raspberry Pi website on your computer. Unzip all files. Transfer all files onto the SD card using the micro SD card reader. After this is done, insert the SD card carefully into the Raspberry Pi. Turn on a monitor, plug the Raspberry Pi to a monitor. Plug in a keyboard and a mouse into the USB hub, and the hub into the Pi. Then and only then, plug in the power chord into the Pi. It will turn on automatically.

Follow the instructions on the screen to install Raspbian Jessie, which is the most recent OS image.

The Real Time Clock (RTC)

First, solder the male header strip to the Pi zero (Supplementary Figure 1); let the board cool down after soldering a few pins, or it may overheat. Then, cut four flexible electric wires (different colours make it easier for the next steps), strip and tin their ends (Supplementary Figure 2). Solder one cable to each of the following holes on the RTC assembled breakout board (Supplementary Figure 2B and C): GND (black), 5V (red), SDA (blue) and SCL (yellow); leave SQW untouched.

With pliers, cut a 1x2 and a 1x3 section (or one 2x3) of the GPIO female header (Supplementary Figure 3A), and tin the pins (Supplementary Figure 3B). Solder the GND and 5V wires to the 1x3, leaving the middle pin untouched (Supplementary Figure 3C). Solder the SDA and SCL wires to the 1x2 section (Supplementary Figure 3D). Then, insert the pins of the Raspberry Pi into these GPIO

female header (Supplementary Figure 3E). Also see the distribution of the Pi Zero pins on Supplementary Figure 4. The RTC board can then be attached to the Pi's protector with some Velcro.

To configure the RTC, follow the instructions provided on the Adafruit tutorials here and then here. The wifi dongle has to be connected to the Pi in order to set the time precisely. While configuring the RTC, its battery should be inserted. The time zone of the RTC can be changed by opening a terminal, typing in `sudo raspi-config`, and then follow `Internationalization`.

The camera

The camera will come with a camera cable that is only suitable for larger models of the Raspberry Pi. Thus, it needs to be unplugged. To do so, gently pull the black piece of plastic that is maintaining the cable connected to the camera. Pull on both sides simultaneously and be careful not to pull it off. The cable should slide out of the slit. Replace it with a cable compatible with the Pi Zero v1.3. Connect the cable to the Pi Zero while the latter is off. When this is done, turn on the Pi and in a terminal, type in `sudo raspi-config` and follow `Enable the camera`.

Camera holder

The camera holder has to be laser-cut in a piece of 1/4"-thick cast acrylic. The holders for both an Allentown mouse and rat cage were designed in Autodesk Inventor Professional 2016 (http://www.autodesk.com). The drawings are provided as separate files ("CameraHolder_MouseCage.dxf", "CameraHolder_RatCage.dxf"). In the cut-out piece of acrylic, insert the fisheye lens. Depending on the model, a file will have to be used to slightly increase the size of the hole. Test it regularly after a bit of filing, until the lens fits.

For the camera to be attached to this piece, two 2-56 screws need to be used. Do not screw them all the way in, leave ~2mm, otherwise the camera will be tilted. Secure the screws with two hex nuts.

Use a 1-18 screw in the lower hole of the holder, it will later be useful to adjust the angle of the camera relative to the cage wall.

LED strip

To provide enough IR light to the camera, you need to prepare an LED strip. Three LEDs are enough for both a mouse cage and a rat cage, but you need to jump all the resistors by soldering a small piece of wire onto them (Supplementary Figure 5A). The strip also needs a connector cable. To connect the LED strip to the Pi, it is necessary to solder the connector cable to a USB cable. To do so, strip the USB cable, cut the green and white wires short, but at different lengths to avoid contact. Strip the red and black wires. Slide one large heat-shrinkable tube around the whole USB cable. Strip the connector cable (both red and black parts). Slide each wire into a smaller heat-shrinkable tube. Tin and solder the ends of the connector cable to the ends of the USB cable. After this is done, heat-shrink the small tubes, and then the larger tube on top, to protect the connection (Supplementary Figure 5B).

You will then be able to plug in the LED strip to the USB OTG cable (Supplementary Figure 5C), and to the Pi zero. Check that the LEDs work. They should provide enough light for a mouse or a rat cage.

Assembly

The Pi zero should be on top of the cage's lid. The camera cable should come towards the cage card holder, be slid into the slit between the green rectangle and the metallic piece of the card holder (Supplementary Figure 6A), and then connected to the camera (Supplementary Figure 6B and C). This is a delicate procedure. The camera setup should be attached to the card holder. Then you can adjust the angle of the camera with the long 1-18 screw (Supplementary Figure 6D and E).

To start up the Pi, simply plug in the Yoobao battery pack.

Programming the Pi to record at a given time

First, create a folder on the desktop called "Mating_monitoring". Then, on the desktop, paste the python script provided as a separate file ("Mating_monitoring.py"). This will record 8hrs 15mins of videos in 5-minute chunks and store them in a .h264 format in the Mating_monitoring folder. You can read this format with VLC (http://www.videolan.org/vlc), or you can convert them to .mp4 with these extra lines to the python script:

```
conversion = "MP4Box -fps 30 -add xxxx.h264 xxxx.mp4"
call ([conversion], shell=True)
deletion = "rm xxxx.h264" # optional if  you want to delete the .h264 video and keep only the converted file
call ([deletion], shell=True)
```

Then, open a terminal, and type in:

```
crontab -e
```

This will open a file that allows to program the launching of scripts at given time points. Instructions are given in the file. At the bottom of the file, type in, for example:

```
55 13 * * * python home/pi/Desktop/Mating_monitoring.py
15 22 * * * sudo shutdown -h now
```

The first line launches the python script that will record videos at 13:55. The second line allows to shutdown the Pi once the recording is done, at 22:15. It is better for the lifespan of the SD card to shut down by a command than by running out of battery.

Recording and storing videos

Once the python script, the desktop folder and the `crontab` are ready, you will just have to plug in the Pi once it is set up on a cage. The recording will start as specified in `crontab`.

Once the recording is done, you can unplug the Pi, take out the SD card, slide it in a Raspberry Pi 3 (faster than Pi Zero when transferring a large amount of files) previously connected to a monitor. Plug in the Raspberry Pi 3 and transfer the videos from the Mating_monitoring folder onto a USB stick or external hard drive.

PARENTAL BEHAVIOUR TEST: PRELIMINARY DATA

A first version of the parental behaviour test was performed on a few mice to obtain preliminary data (data enclosed). Some tests were performed during the light phase, and some during the dark phase. The behaviours of four mice during the light vs. dark phases are displayed on Supplementary Figure 7. No major difference was observed between the two phases, although parental behaviours seemed slightly more intense during the light phase (longer time spent huddling, higher proportion of times the pup was retrieved, shorter latency of physical contact after first approach). Thus and to be consistent with Bendesky et al. (2017), it was decided that this test would be performed during the light phase, while the test of attraction/aversion to a pup would be done in the dark phase.

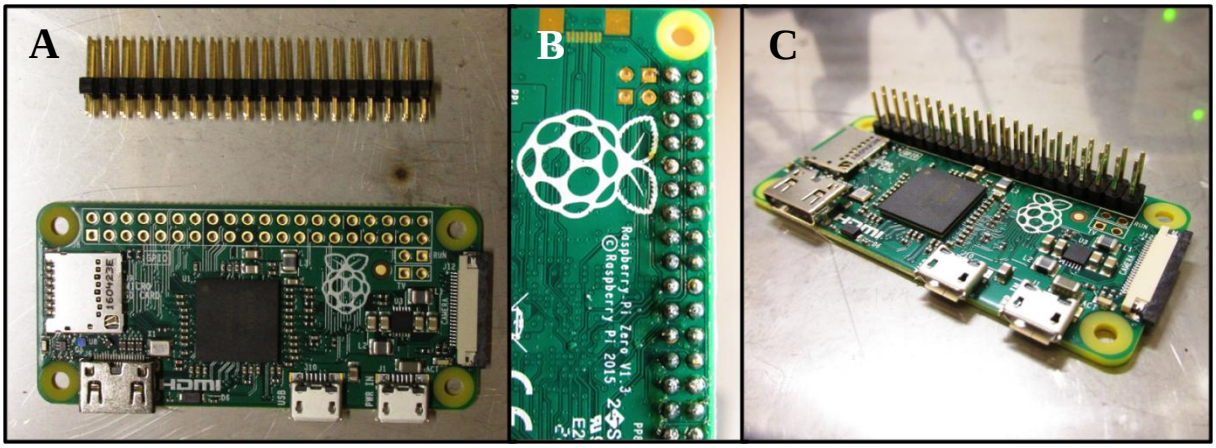
TEST OF ATTRACTION/AVERSION TO A PUP: DECISION OF DURATION

The initial duration of the test of attraction/aversion to a pup was arbitrarily set to 20 minutes. After partial collection of the data, I compared the data of six *Peromyscus maniculatus bairdii* (*P. maniculatus* hereafter) virgin males to those of six *Peromyscus polionotus subgriseus* (*P. polionotus* hereafter) mothers, presumably extreme in their reactions to the test. I hypothesized that *P. maniculatus* virgin males would be deterred by/indifferent to the pup, while *P. polionotus* mothers would be strongly attracted to the pup.

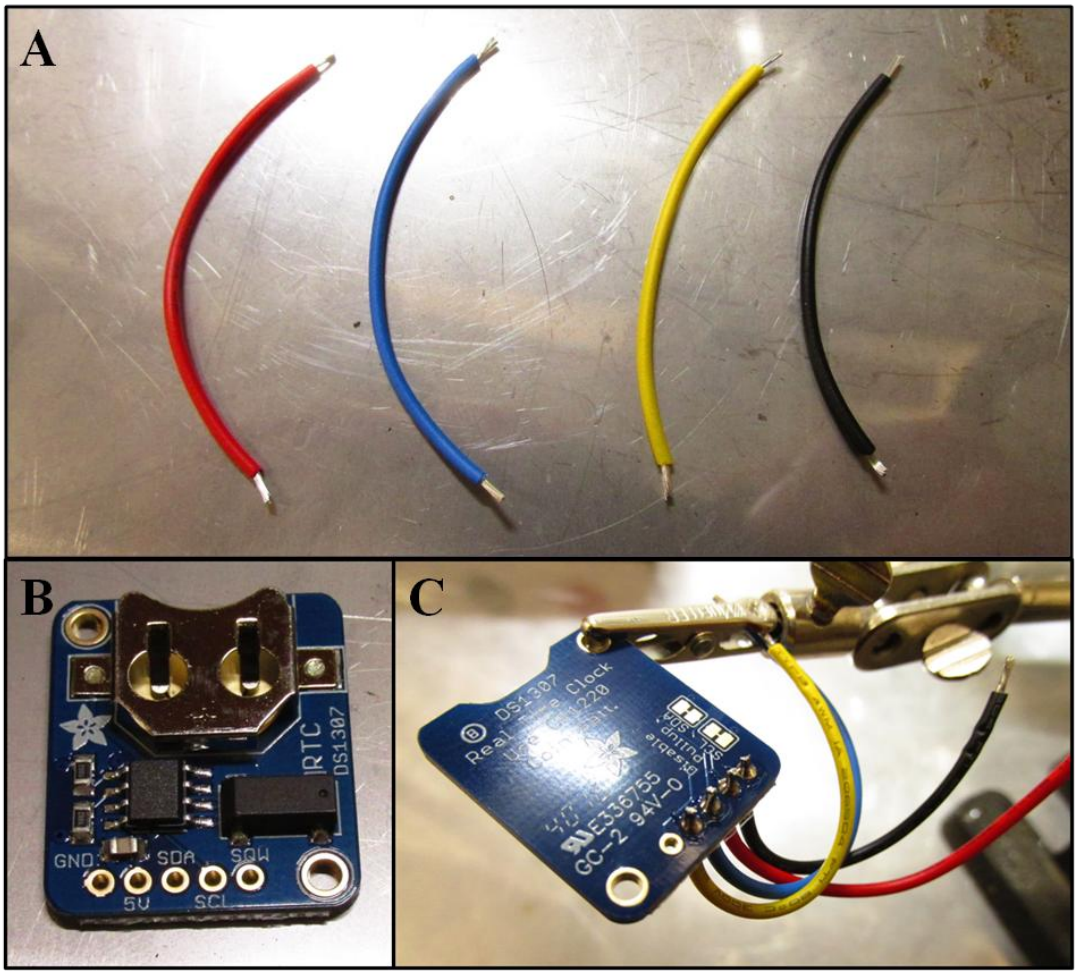
In an attempt to investigate which part of the 20 minutes was the most informative, I compared the proportion of time spent in each zone (Supplementary Figure 8A and B) and the pup VS. peanut butter preference index (see main report to see how the latter was calculated; Supplementary Figure 8C) between the first and the second half of the test (from t=1'00" to 10'30") and from t=10'30" to 20'00", respectively; Supplementary Figure 8). As a complementary visual assessment of the data, I generated heat maps using Ethovision XT 11.5 (Noldus et al. 2001) and looked at individual data for the 12 mice of interest. The raw data is enclosed as a separate file ("Attraction-aversion_duration.xlsx"). Exemplary heat maps of a *P. maniculatus* virgin male and a *P. polionotus* female are shown for each half of the test on Supplementary Figure 9.

From these figures, it appeared that the second half of the test did not substantially amplify the behavioural difference between *P. maniculatus* virgin males and *P. polionotus* mothers. As a result and for practical reasons, I decided to shorten the test duration to 10 minutes.

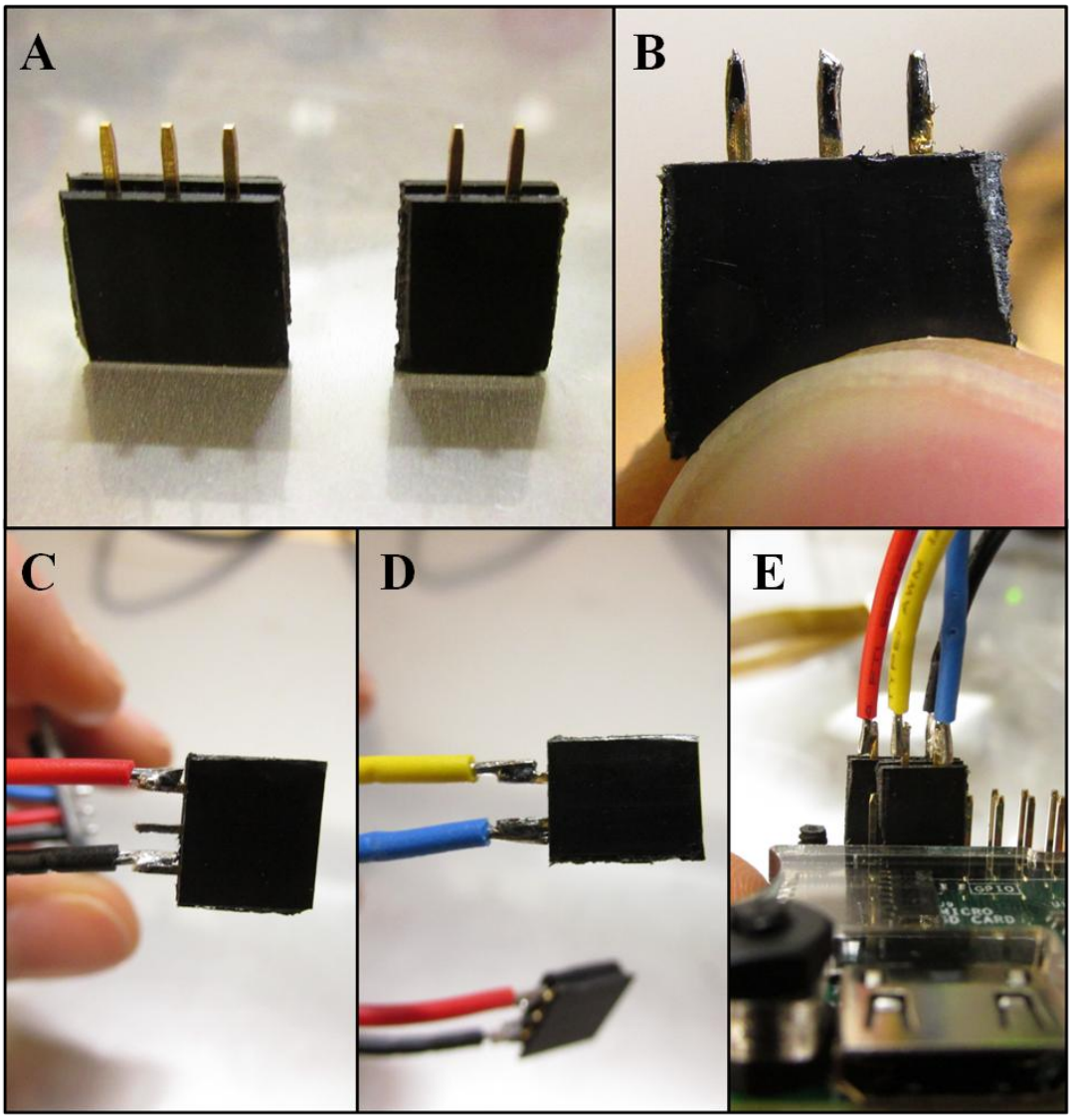
SUPPLEMENTARY FIGURES



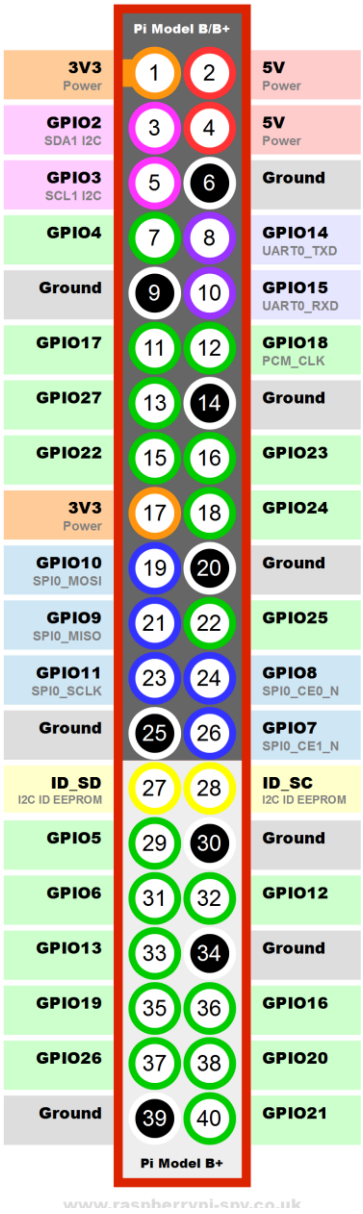
Supplementary Figure 1 - Raspberry Pi Zero and soldering of the male header strip. (A) Raspberry Pi Zero from above and male header strip before soldering. (B) Raspberry Pi Zero from below with soldered male header strip. (C) Raspberry Pi Zero with soldered male header strip.



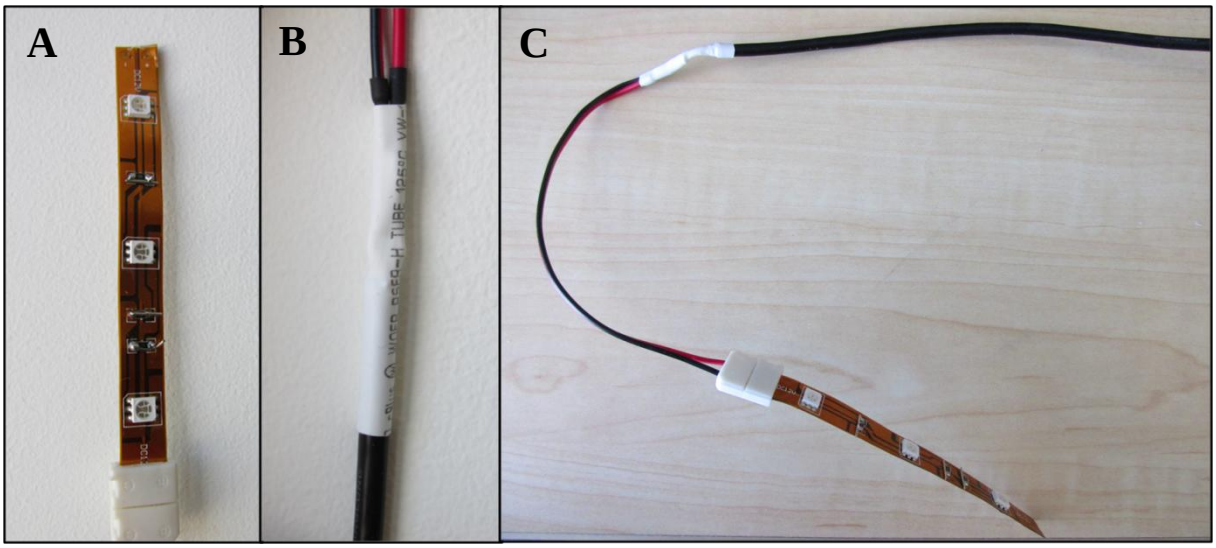
Supplementary Figure 2 - Flexible electric wires of different colours and soldering to the Real Time Clock (RTC) assembled breakout board. (A) The ends of the electric wires are stripped and tinned. (B) RTC assembled breakout board. (C) Soldering of each colour electric wire to the corresponding hole on the RTC assembled breakout board.



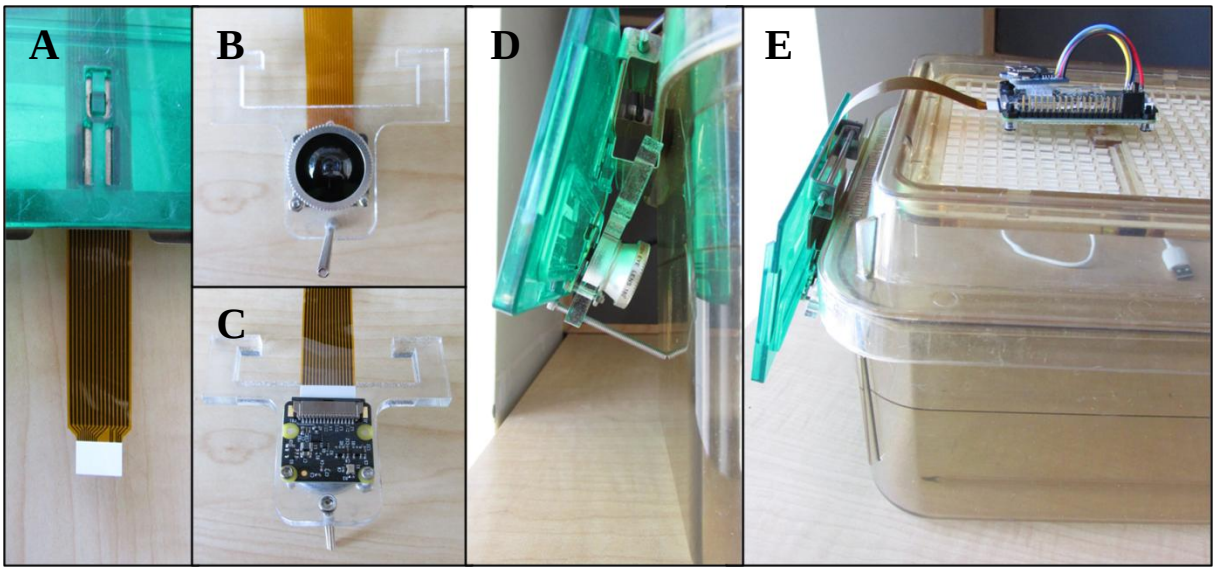
Supplementary Figure 3 - GPIO female header and soldering to the electric wires. (A) One 1x3 and one 1x2 section of a GPIO female header. (B) Tinned pins of the 1x3 section. (C) Soldering of the 1x3 section to the red wire (5V) and to the black wire (GND). (D) Soldering of the 1x2 section to the yellow wire (SCL) and to the blue wire (SDA). Assembly of the electric wires and the GPIO female header to the Raspberry Pi Zero pins.



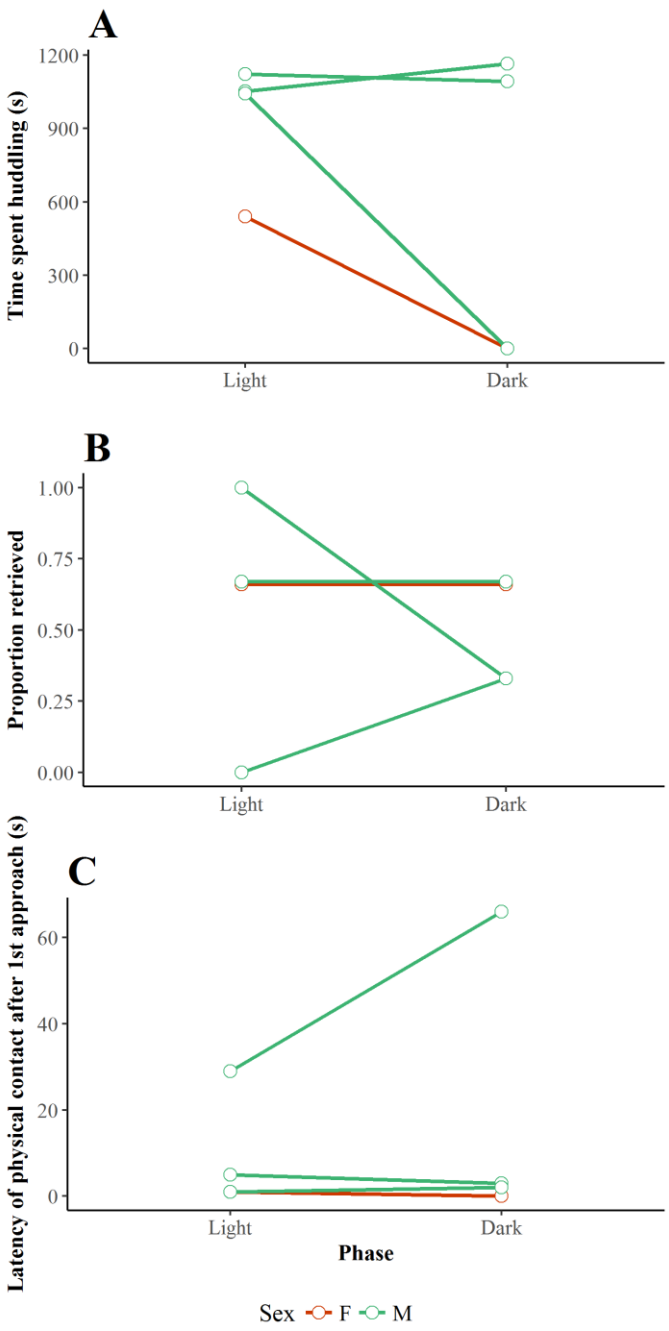
Supplementary Figure 4 - Pin distribution of the Raspberry Pi models 3B and Zero from [this website](#). The pins that are of use in this tutorial are 5V, Ground (GND), GPIO2 SDA I2C (SDA) and GPIO3 SCL1 I2C (SCL).



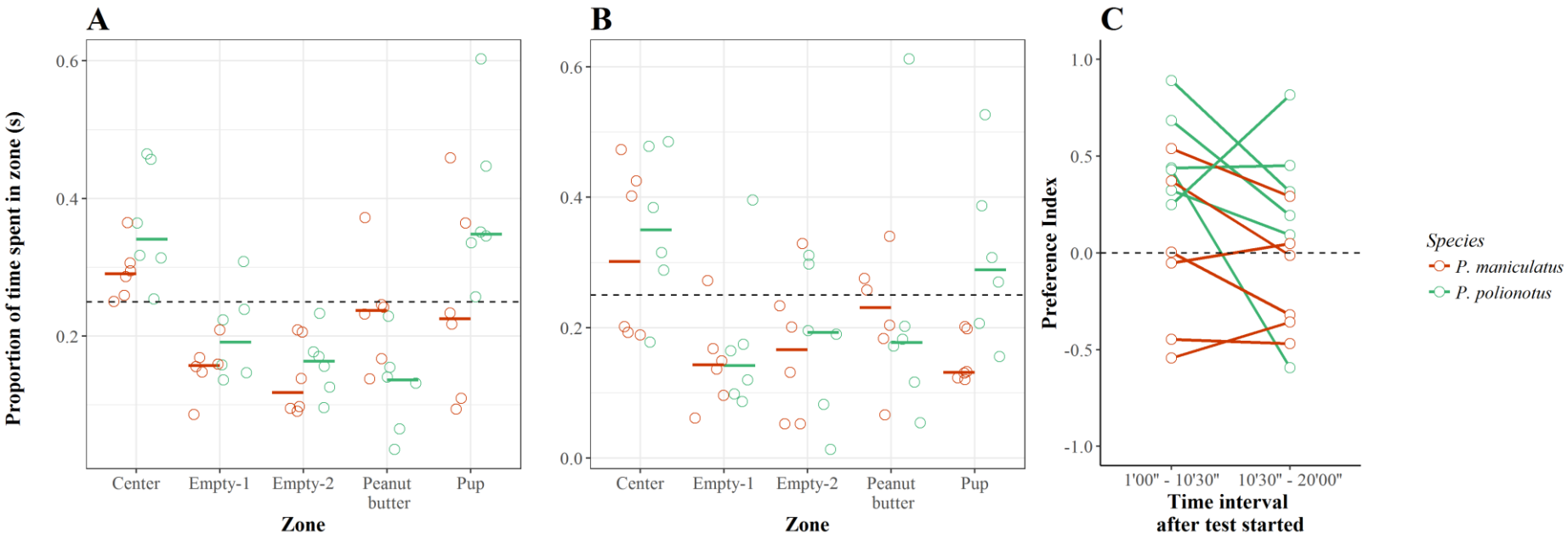
Supplementary Figure 5 - Modification of an IR LED strip to illuminate the cage. (A) LED strip with jumped resistors. (B) Heat-shrinkable tubes connecting the USB cable to the connector cable. (C) Final assembly of the LED strip and USB cable.



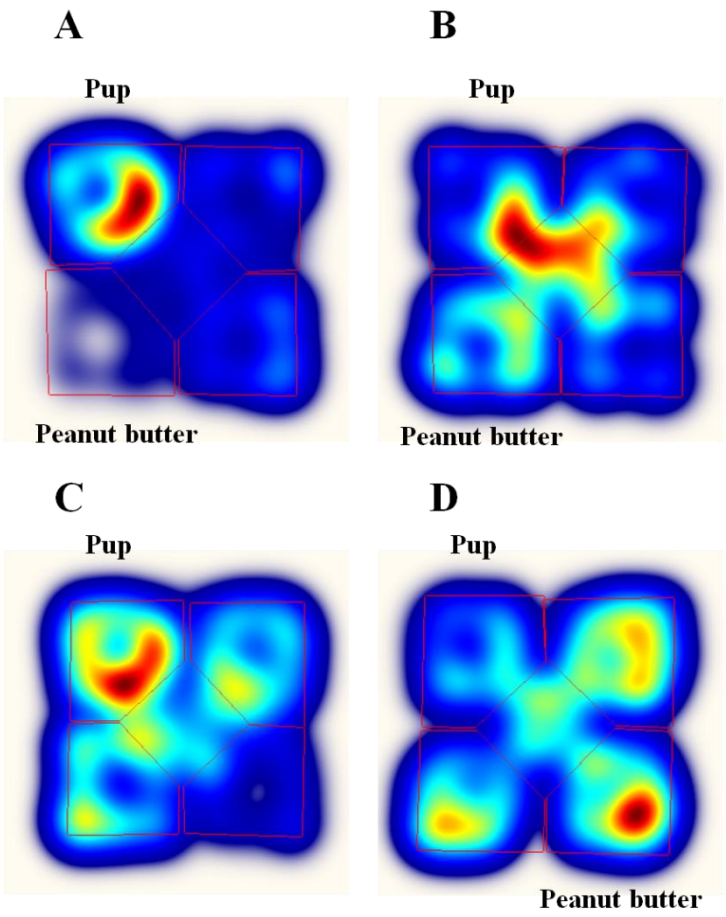
Supplementary Figure 6 - Final assembly of the camera set up on a mouse cage. (A) The camera cable goes into the slit of the cage card holder. (B) Assembled camera, fisheye lens, camera cable and screw (front view). (C) camera, fisheye lens, camera cable and screw (back view). (D) Final assembly on a mouse cage, the long screw allows to adjust the angle of the camera (side view). (E) Final assembly on a mouse cage, the Raspberry Pi Zero is on top of the cage, the camera cable is connected to the Pi and to the camera. The battery pack is not on the picture.



Supplementary Figure 7 - Preliminary data for the parental behaviour test. Some behaviours displayed for four individual parent mice (three males, one female) born from a *P. maniculatus* x *P. polionotus* cross. A mouse was always tested during the light phase first, and never tested twice on the same day.(A) Time spent huddling. (B) Proportion of time the pup was retrieved (out of 3 times). (C) Latency of physical contact (handling, licking, huddling or attack) after first approach.



Supplementary Figure 8 - Data for the test of attraction/aversion to a pup from six *P. maniculatus* virgin males and six *P. polionotus* mothers. (A) Proportion of time spent in each zone of the arena during the first half of the test (from 1'00" to 10'30"). The dashed line represents the expected proportion of time spent in one of the peripheral compartments if mice were moving uniformly across zones. (B) Proportion of time spent in each zone of the arena during the second half of the test (from 10'30" to 20'00"). The dashed line represents the expected proportion of time spent in one of the peripheral compartments if mice were moving uniformly across zones. (C) Preference index in the first and second half of the test. The dashed line represents the preference index of an individual who spent as much time in the pup zone as in the peanut butter zone.



Supplementary Figure 9 - Exemplary heat maps drawn with Ethovision XT 11.5. Warmer colours represent longer amounts of time spent in a given area, colder colours represent shorter amounts of time. The zones containing a pup/peanut butter are labeled. (A) Example *P. polionotus* mother in first half of the test (1'00"-10'30"). (B) Example *P. maniculatus* virgin male in first half of the test (1'00"-10'30"). (C) Example *P. polionotus* mother in second half of the test (10'30"-20'00"). (D) Example *P. maniculatus* virgin male in second half of the test (10'30"-20'00").

16 **LITERATURE CITED**

17 Bendesky A, Kwon Y-M, Lassance J-M, et al (2017) The genetic basis of parental care evolution in
18 monogamous mice. Nature 544:434–439.

19 Noldus LPJJ, Spink AJ, H RAJT (2001) EthoVision : A versatile video tracking system for automation of
20 behavioral experiments. Behav Res Instruments, Methods Comput 33:398–414.

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