Single Entry Module

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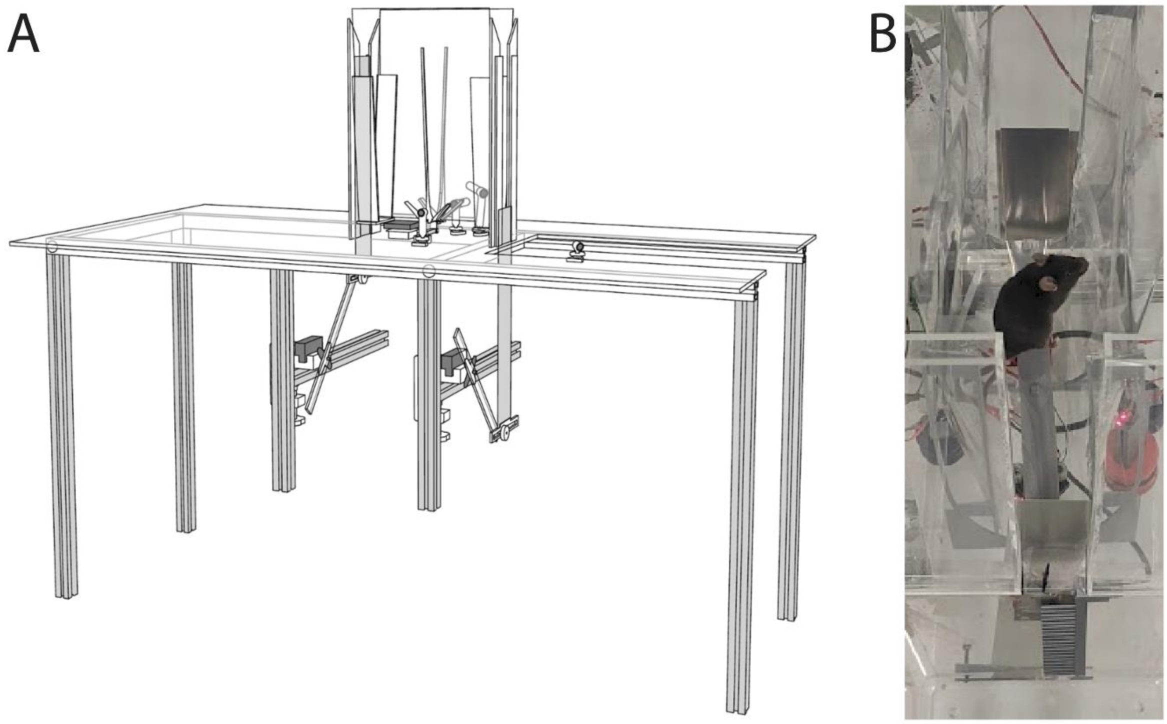

Figure 1. The single entry module. A, CAD model. B, Picture of a mouse passing through the apparatus.

General description

The following build instructions are for a generic 'single entry module' (SEM) which we have incorporated in larger builds not explained here. These reduced instructions illustrate the build of a device that connects an empty slot for a mouse cage (Figure 1A right side) to an open area (Figure 1A left side). For actual experiments, the SEM needs to be installed together with a behavioral measurement device, such as for example an open field arena, in place of the open area to which the mouse is passed.

The SEM (Figure 1) consists of a 200 mm long U-shaped corridor, constructed from a sheet of transparent, firm, light-weight plastic (made from two overhead projector transparency sheets) which is secured into a U-shape via a 3D-printed skeleton (PLA). The U-shaped corridor is screwed onto a 100 g load cell (SparkFun TAL221 SEN-14727) which is secured to a large acrylic floor plate. The load cell is connected to a USB scale (SparkFun SEN-13261), which is positioned next to the SEM. Laser cut 3 mm acrylic sheet pieces are used to construct edge guides for the two doors by chemical welding using chloroform (using appropriate PPE). Additional pieces of acrylic sheet are attached to the door edge guides to support the edges of the U-shaped corridor in case an animal pushes against it. The SEM can be closed off on both ends via vertical sliding doors constructed from 300 mm X 50 mm rectangles of 0.5 mm thick aluminum sheet which can be lowered into the floor of the setup via a servo motor attached to a beam below. An RFID-tag reader (Sparkfun ID-20LA and SEN-09963) is placed below the U-shaped corridor, near door 2. Two beam break devices (BB), constructed from a 650 nm laser diode (SparkFun P1054) and photoresistor (Sparkfun SEN-09088) circuit mounted on 3D-printed ball joints are used to ensure safe operation of the doors. One (BB1) is directed 10 mm above door 1 in open position. It is used to prevent door 1 closing with an animal on top. This beam is unbroken when door 1 is open and no animal is sitting in the opening. Another (BB2) is directed through the corridor at a safe distance (>100 mm) from door 2. A break in this beam signals exit through the module back to the home cage.

Operation of the module according to Raspberry Pi code *SEM_only.py*:

MODE 1 SEM is open for entry from home cage.

MODE 2 An animal is detected with the RFID reader. If the weight is in range of one animal and no animal is detected on top of door 1, door 1 is closed, trapping one animal in the SEM. Otherwise wait for next RFID detection.

MODE 3 The weight is determined by averaging 50 consecutive reads. If the weight is in range of one animal, door 2 is opened and a minimum time out (minimum_entry_time) is waited until exit detection ensues.

MODE 4 When the animal is returning to the home cage through the SEM, BB2 is broken, and consequently door 2 closes, door 1 opens and the SEM is open for the next entry (MODE 1).

Materials

Table 1. Bill of materials for the SEM.

Part	Count, vol. or length	Manufacturer/ Supplier	Manufacturer serial # or *.stl file	Approximate cost, EUR	Part
Arduino nano	1	Arduino	A000005	19.9	
Raspberry Pi 4b	1	Raspberry Pi	KW-2646	45 – 95.0	
Servo motor	2	Master	1556176 - 62	56.0	
Switch	1	Adafruit	P3064	2.9	
5V power supply	2	RS Pro	124-2183	23.0	
wire	500 mm	RS Pro	196-4225	0.2	
2-core shielded cable	2000 mm	Lapp	0034302	1.0	
5kOhm resistor	2	Yageo	FC0204JT-52-5K	0.2	
Breadboard	1	Bud industries	BB-32650-B	3.1	
Load cell 100 g	1	SparkFun	TAL221	9.4	

			SEN-14727		
Open scale	1	SparkFun	SEN-13261	32.5	
RFID antenna	1	SparkFun	ID-20LA SEN-11828	33.7	
RFID USB reader	1	SparkFun	SEN-09963	26.3	
Mini USB cable	2	SparkFun	CAB-13243	4.0	
650nm Laser Diode	2	SparkFun	P1054	11.2	
Photoresistor	2	SparkFun	SEN-09088	1.6	
Female to female jumper wire connectors	4	MikroElectronika	MIKROE-511	4.3	
Male to male jumper wire connectors	4	MikroElectronika	MIKROE-513	4.3	
Transparent film A4 pieces	2	Staedtler	636 10DT6F	0.4	
Chloroform*	1 ml	Sigma-Aldrich	288306	0.5	
300 mm X 50 mm rectangles of 0.5 mm thick aluminium sheet	2	Reely	297895 - 8J	8.0	door
3-d printed parts	1		scale1.stl	7.6 all parts	A
	1		scale2.stl		B
	1		scale3.stl		C
	2		scale4.stl		D
	4		bb_base.stl		E
	4		bb_base_nut.stl		F
	1		bb1_send.stl		G
	1		bb1_receive.stl		H
	1		bb2_send.stl		I
	1		bb2_receive.stl		J
	2		door_base.stl		K
	2		door_base_nut.stl		L
	2		servo_clamp.stl		M
	2		servo_bolt.stl		N
	2		servo_bolt_cap.stl		O
	1		rfid_base.stl		P
3 mm Acrylic sheet parts	4		door_guide_back.svg		Q
	4		door_guide_spacer.svg		R
	4		sem_support.svg		S
	1		floor.svg		T
	2		door_lever.svg		U
1 mm Acrylic sheet parts	4		door_guide_front.svg		V

	2		prevent_burrowing.svg		W
20 mm square profile aluminum rail, 500 mm length	4	McMaster-Carr	5537T101	64.2 all profile parts	
20 mm square profile aluminum rail, 300 mm length	6	McMaster-Carr			
20 mm square profile aluminum rail, 1000 mm length	2	McMaster-Carr			
M6 cap screws, 30 mm length	6	McMaster-Carr	90128A266	9.2	
M6 grub/set screws, 30 mm length	8	McMaster-Carr	92015A136	6.5	
M6 Drop-In T-Nut	8	Thorlabs	XE25T1/M	30.5	
M3 cap screws, 30 mm length	4	McMaster-Carr	91290A171	7.2	
M3 cap screws, 10 mm length	2	McMaster-Carr	91274A105	6.4	
M3 nuts	2	McMaster-Carr	90592A085	2.6	

Approx. total cost: 421.7 - 471.7 EUR.

* Welding acrylic and 3D-printed PLA parts together with chloroform must be done safely in a well ventilated space, with minimal amounts of chloroform and using appropriate PPE including gloves and an appropriate mask.

The 3D-printed and laser cut part drawings can be found at:
https://github.com/MaheshKarnani/Switch_maze/tree/main/Modules_SM/SingleEntryModule

Useful tools: Drill, Allen key set, a small screw driver, wire cutters, hacksaw, metal shears, metal file, a soldering iron, tape, 1 ml syringe for the chloroform, personal protective equipment and epoxy glue.

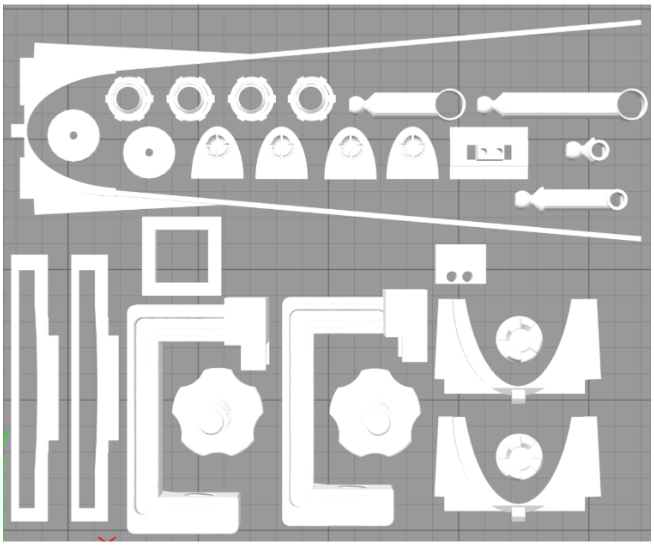

Figure 2, 3D printed parts on the build plate.

Mechanical assembly

1) Elevated floor frame:

Build a support frame for the floor plate using 2x 300 mm, 2x 1000 mm and 4x 500 mm long 20 mm square profile aluminum rail according to Figure 3 and lay the 3mm acrylic sheet piece (floor.svg) on top (part S). Use a drill with a 6 mm drill piece suitable for aluminum to make holes through the 1000 mm rails for the 300 mm cross beams. Steel 6 mm screws will readily form a thread in the axial hole of the rails when first screwed in, but this is best done before assembly as some force may be required. Use drop-in nuts and grub screws for the 500 mm legs. A minimum height of 500 mm from the ground is recommended as the doors need to operate some distance below the floor sheet.

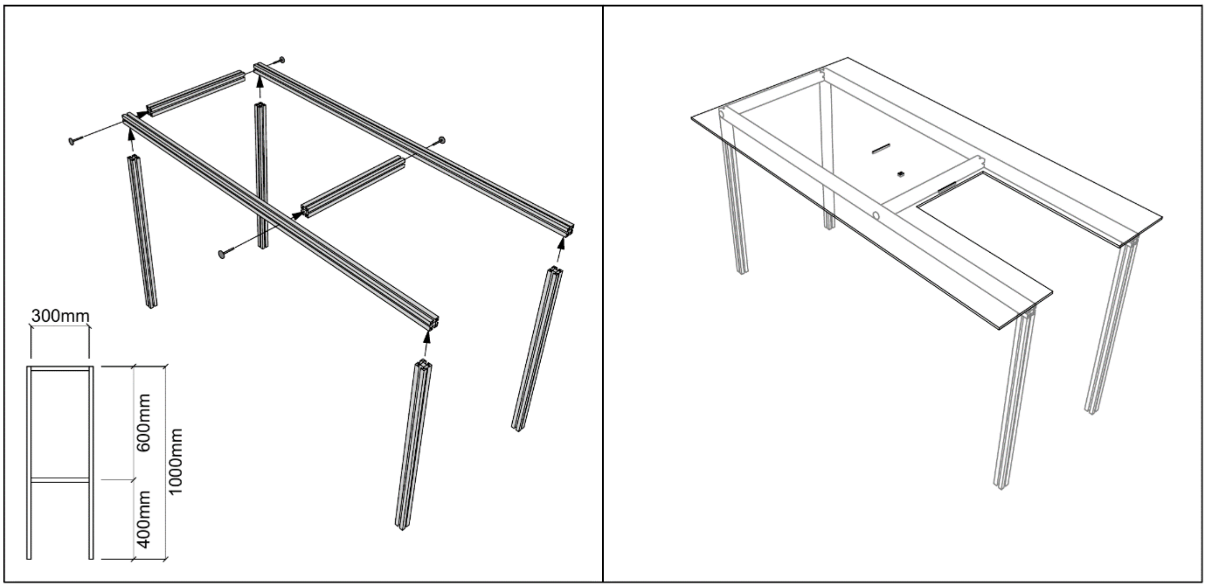

Figure 3. Aluminum rail frame and acrylic floor sheet.

2) U-shaped corridor with RFID detector and scale:

Four guide frames for the doors are constructed using acrylic sheet pieces P, Q and U (Figure 4A). Clamp each set of three pieces together with a binder clip (Figure 4B) and use a drop of chloroform to bond, making sure the three pieces are aligned at the bottom for a strong bond to the floor plate. Each frame forms a 3 mm slot that functions as a rail for one side of a door. Make sure to align these slots with the 3 mm door aperture in the floor plate, and to leave enough room for the door to slide up and down while remaining within the guide frame (Figure 4C). Repeat on the other edge of the door. Build frames for both doors.

The RFID detector (SEN-11828 connected to SEN-09963) is attached to the square shaped 3D-printed support (rfid_base.stl, part P) using two small drops of superglue, and this assembly is placed near door 2, leaving a minimum of 25 mm clearance between the door frame and RFID detector (Figure 4D). The support can then be joined to the floor plate with a drop of chloroform.

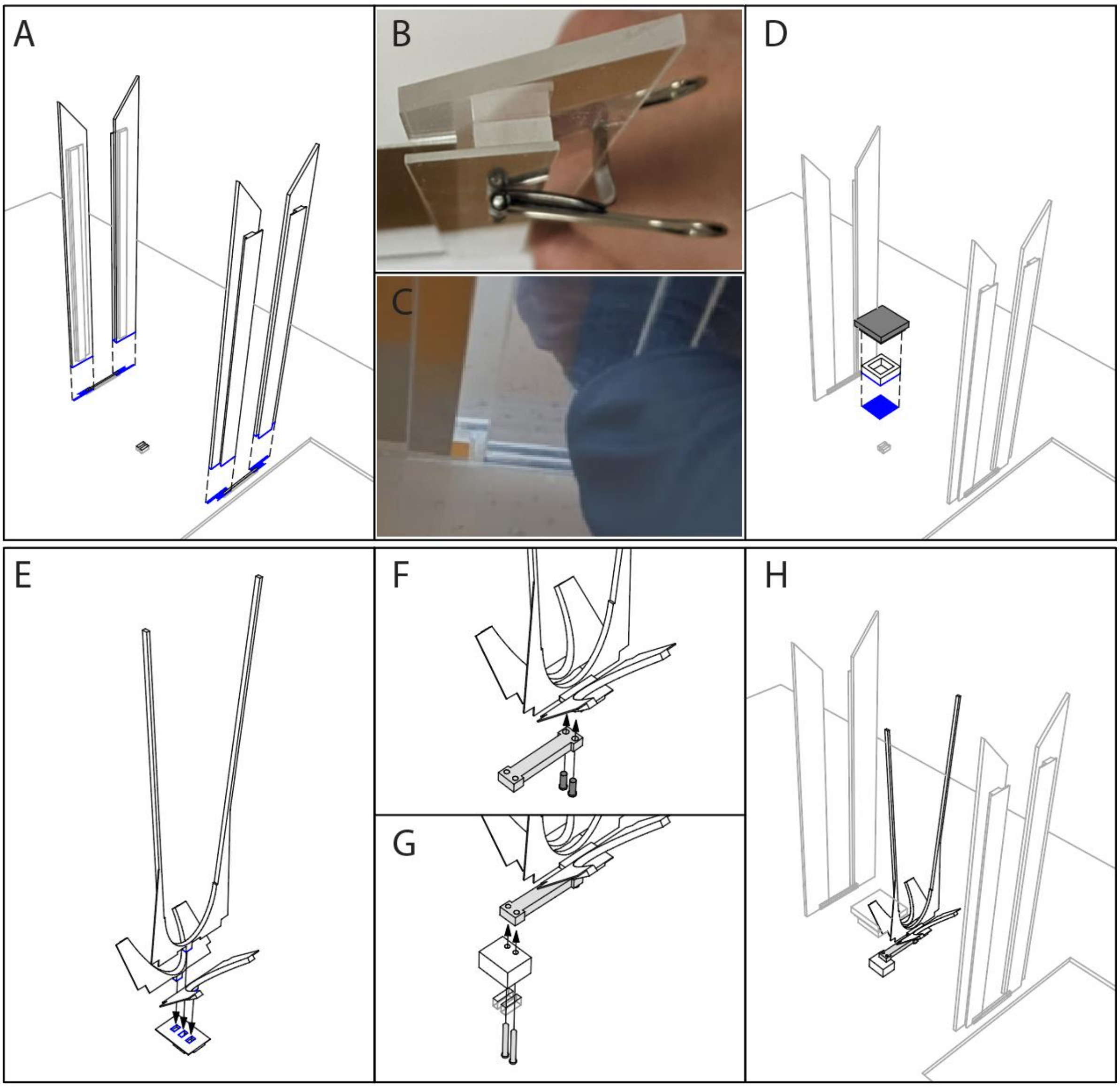

Figure 4. Guide frame, RFID detector and scale backbone installation. Use PPE and minimal amounts of chloroform.

After guide frames for both doors are built, the U-shaped corridor with the scale is assembled as follows. A scale backbone is assembled from 3D-printed parts A-D and a 100 g load cell (Sparkfun SEN-14727) by push-fitting the pieces as shown in Figure 4E and securing it to the loadcell using M3 screws (Figure 4F). The prints may need to be filed down at the attachment points to get a good fit. A loose push-fit is acceptable at this stage as a solid joint will be made with epoxy-glue at a later stage. The complete scale backbone is screwed in place firmly with two 30 mm M3 screws passed through the 10 x 3 mm slits in the floor plate and 3D-printed part A. The screws are tightened into the threaded holes on the load cell (Figure 4G).

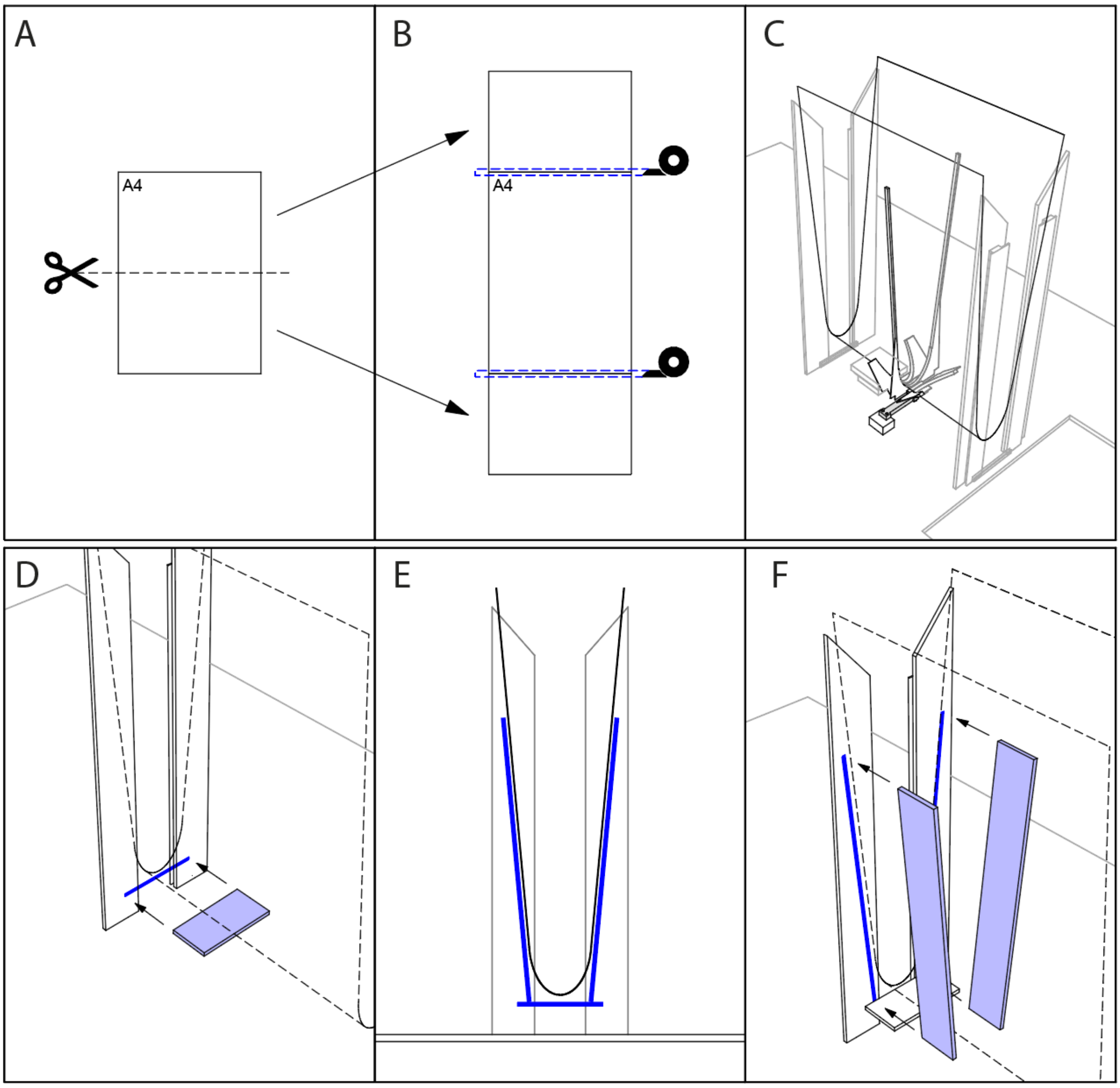

Figure 5. U-shaped corridor and support structures.

A 597 x 200 mm rectangle of transparency film is made from two A4 sheets of film, cutting one in half and taping the halves to the ends of the intact one, followed by cutting 10 mm off the long edge (Figure 5A,B). The film is then bent into a U-shape that fits inside the scale backbone and taped in place along the tall middle supports. It may be necessary to cut the ends slightly to make the film fit snugly in between the doors (Figure 5C). The edges of the film should not push against the door frames, but there should also be no gaps larger than 1-2 mm. A new piece of film is simple to install at this stage if the gaps are too large after cutting. When an acceptable fit is achieved, the film is taped firmly in place along the tall middle supports of the backbone and epoxy glued to the backbone along the U-shaped parts hugging it. Enough epoxy (1-2 ml) should be applied to now also firm up any loose push-fit junctions between the 3D-printed parts. The finished U-shaped corridor should be a solid but light-weight piece that transmits the animal's weight to the load cell regardless of animal movement in the scale.

Next, two 1 mm thick acrylic pieces (prevent_burrowing.svg, part W), that prevent burrowing below the scale, are attached using a drop of chloroform to the door frame guides about 2 mm below the transparent film (Figure 5D). Then four 3 mm thick acrylic pieces (sem_support.svg, part S) are attached to the door frame guides using drops of chloroform, about 1 mm away from the transparent film (Figure 5E,F). The film may lean against these supports toward the top, but not at the bottom. This way the animal's weight will be measured accurately while the film wall appears sturdy enough to discourage excavation.

3) Doors:

To prepare the moving parts (Figure 6), a lever made of 3 mm acrylic sheet (part T) is tightly attached to a servo hub (provided with the servo) using a 10 mm M3 screw. In a well ventilated space/chemical hood, 1-2 drops of chloroform are added onto the joint to activate the surfaces. After a few minutes 2-3 drops of superglue are added onto the joint. After the glue has set, the M3 screw can be removed. A 300 mm X 50 mm rectangle of 0.5 mm thick aluminum sheet (part door) is slotted into a 3D printed base (part K) and 2-3 drops of superglue are added onto the joint. A small amount of epoxy glue can be used to bond these glued joints as they may need to carry high loads if installed at less than ideal angles or if the unit is not kept sufficiently clean. A 30 mm M3 cap screw is secured firmly with a M3 nut to the free end of the lever (see also Figure 7C).

Figure 6. acrylic door lever along with attachment pieces (above) and aluminum door (below).

Using the drop-in T-nuts and M6 grub screws, a 300 mm aluminum rail is hung downward from the support frame, and another 300 mm aluminum rail is attached perpendicular to that (Figure 7A). A servo motor is positioned on top of the perpendicular rail and secured using 3D printed clamp (parts M-O; Figure 7B). The door lever to the servo hub is attached such that its movement arc cannot collide with the floor, i.e., so the 180 degree range of the servo stops before the lever would collide with the floor. The door is inserted through its aperture in the floor and the 30 mm screw of the lever is threaded through the door's base (Figure 7C). Then the screw is capped using the 3D printed nut (part L), but not tightened against the door base (see Figure 7C inset). While the servo is powered off, the door can be tested by manually moving the lever up and down (Figure 7D-F). The lever should operate perpendicular to the door, such that the only part touching the door is the 30 mm screw. If the lever's distance to the door base changes during operation, the servo may need to be aligned better by opening the 3D printed clamp, readjusting the servo position and closing the clamp again. There should be no friction during door operation and the door should remain in the frame slots during the up and down motion. If any friction is noticed, the components can be removed (welded joints can be snapped off) and reinstalled at slightly altered locations. Lubricant use should be minimized as the animals can spread them into the rest of the apparatus, however, minimal amounts of Vaseline can be applied to the 30 mm screw shank and the edges of the door.

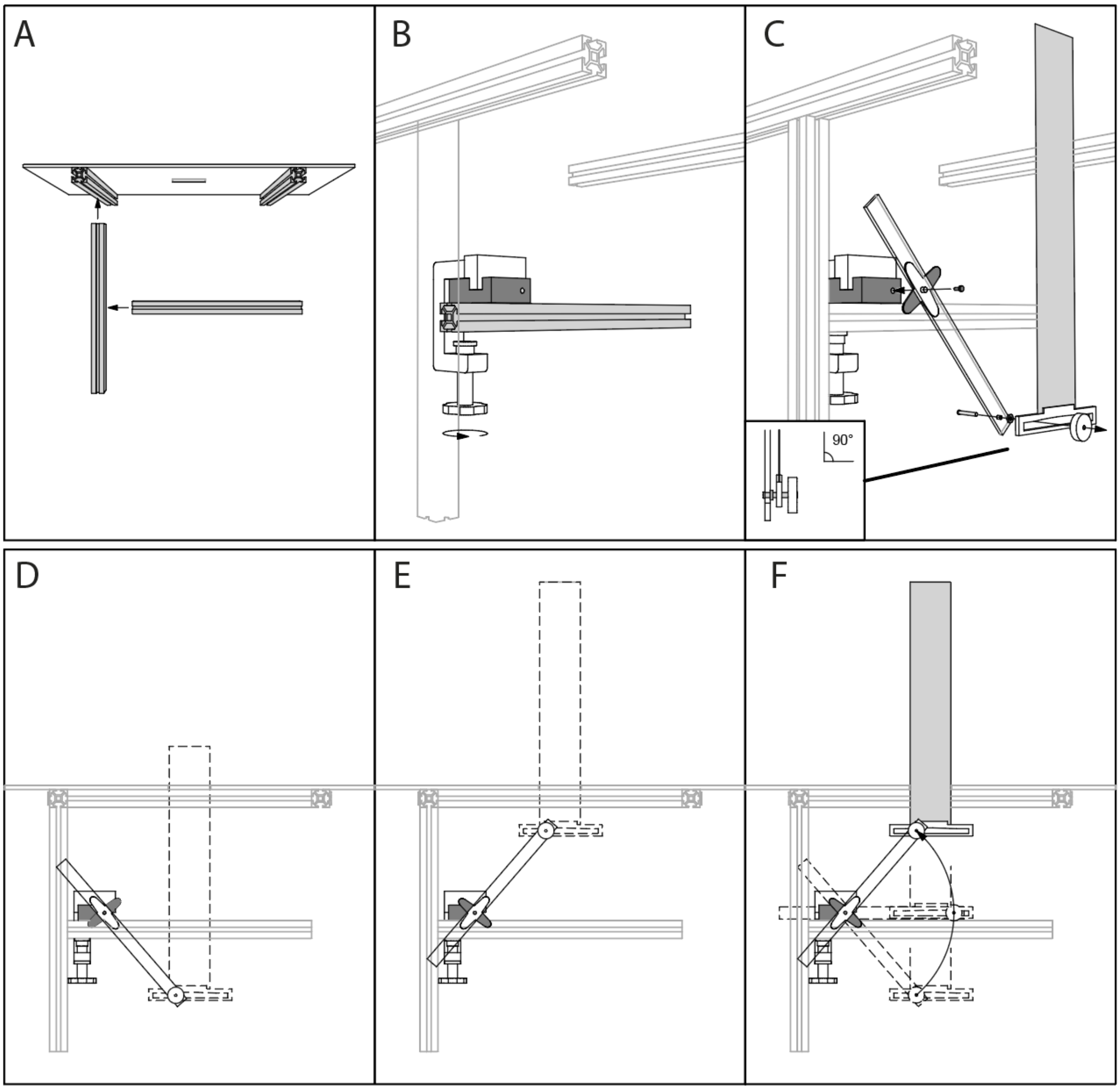

Figure 7. Door installation.

4) Beam break detectors:

Lastly, the beam breaks are installed using 3D-printed parts E-J, diode lasers (Sparkfun P1054) and photoresistors wired to resistors. The laser and detector holders are built first (each has 3 parts: base E, tightening nut F and one of parts G-J with a ball joint). The ball joint may need to be filed smooth depending on the grain of the print. After assembly, the parts are placed at their approximate locations (Figure 8) and door 1 is set to protrude approximately 50 mm from the floor plate (closed position). Then beam targeting is checked by sighting through the laser holders to the detectors. Beam 1 needs to pass in the middle of the entrance about 10 mm above door 1 (Figure 8A,C). Beam 2 needs to pass about 10 mm above the bottom of the U-shaped corridor at a distance of >100 mm from door 2 (Figure 8A,B). A small drop of chloroform is used to weakly bond each holder to the floor plate, so they can be detached if necessary. Later when the lasers and doors are operational, locations can be confirmed and the holders’ base parts should be joined strongly to the floor with additional drops of chloroform.

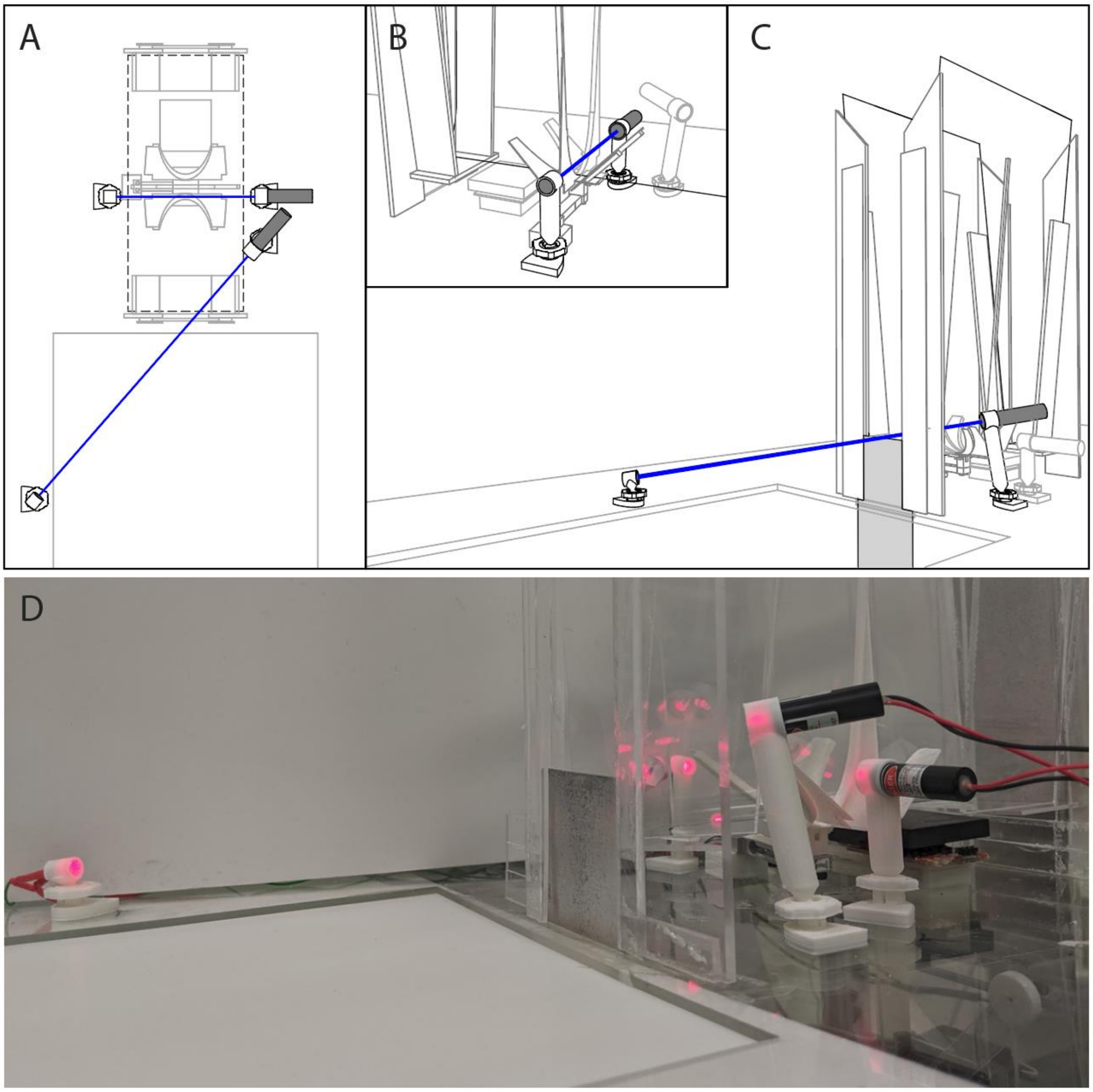

Figure 8. Beam break (BB) devices. BB1 aimed across the top of door 1, and BB2 aimed perpendicularly through the U-shaped corridor.

At this stage the mechanical build is complete and should look like Figure 1.

A Perspex cage bottom measuring about 364*206*138 mm, with a top edge protrusion ‘lip’ (Figure 9) can slide into the 200 mm wide slot on the floor plate. Alternatively a recessed tray with a depth of at least 50 mm can be built from rectangular acrylic sheet pieces. Alternatively the slot of the floor plate can be modified to fit any available cage bottom dimensions before laser cutting it. For our Perspex cage bottom, we use a wall assembly including a 3D printed ladder (Figure 10).

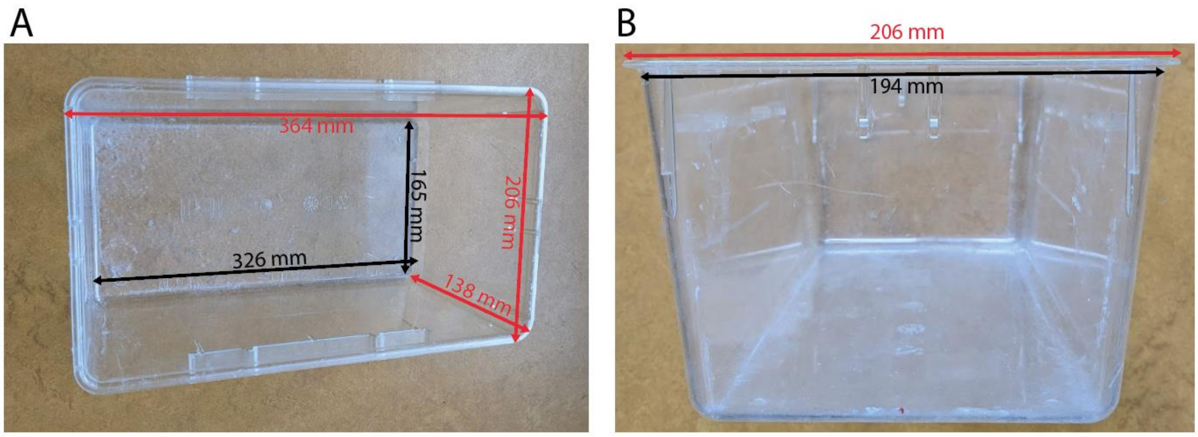

Figure 9. Nest cage bottom.

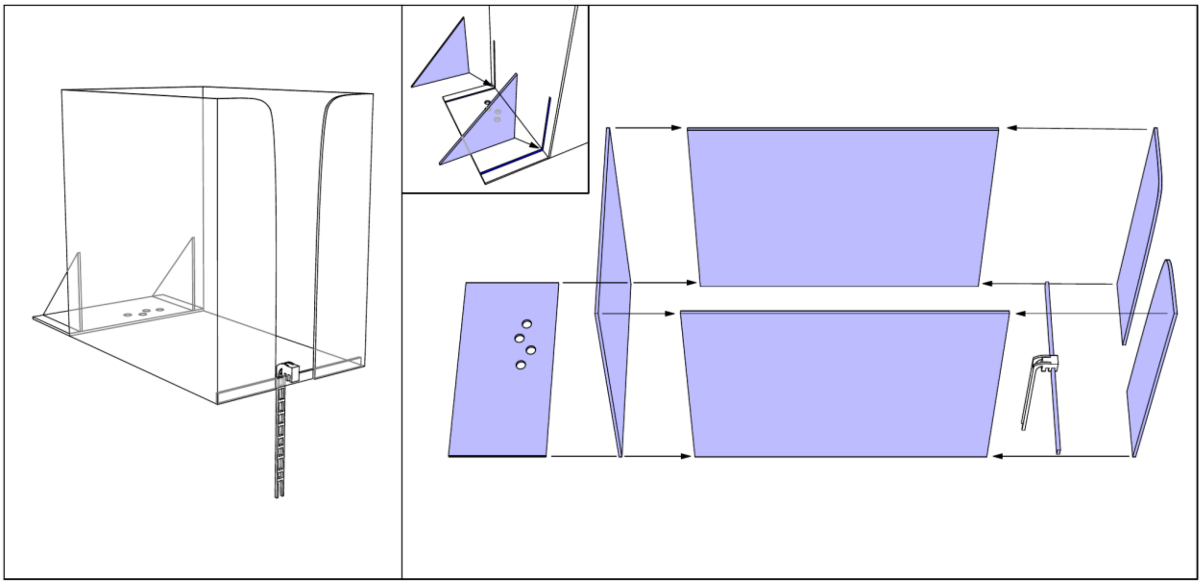

Figure 10. Nest wall assembly including a ladder and holes for different sized water bottles.

Electronics

Two-core shielded cable is used to connect both of the servo motors (Master servo DS6020 C1689) to one 5V DC source through a switch (Adafruit P3064) and a breadboard mounted Arduino Nano. The 650nm Laser Diodes (SparkFun P1054) are connected to a separate 5V DC source (Adafruit P3064) as are both photoresistors (Sparkfun SEN-09088), which also connect to the Arduino Nano across 5kOhm resistors. A raspberry Pi 4b or 400 is connected to the load cell amplifier (SEN-13261) and RFID detector (SEN-09963) via USB cables (USB3.0 ports) and to the Arduino Nano through 5 digital lines. Importantly, all grounds should be wired together.

Connections are made according to Figure 11.

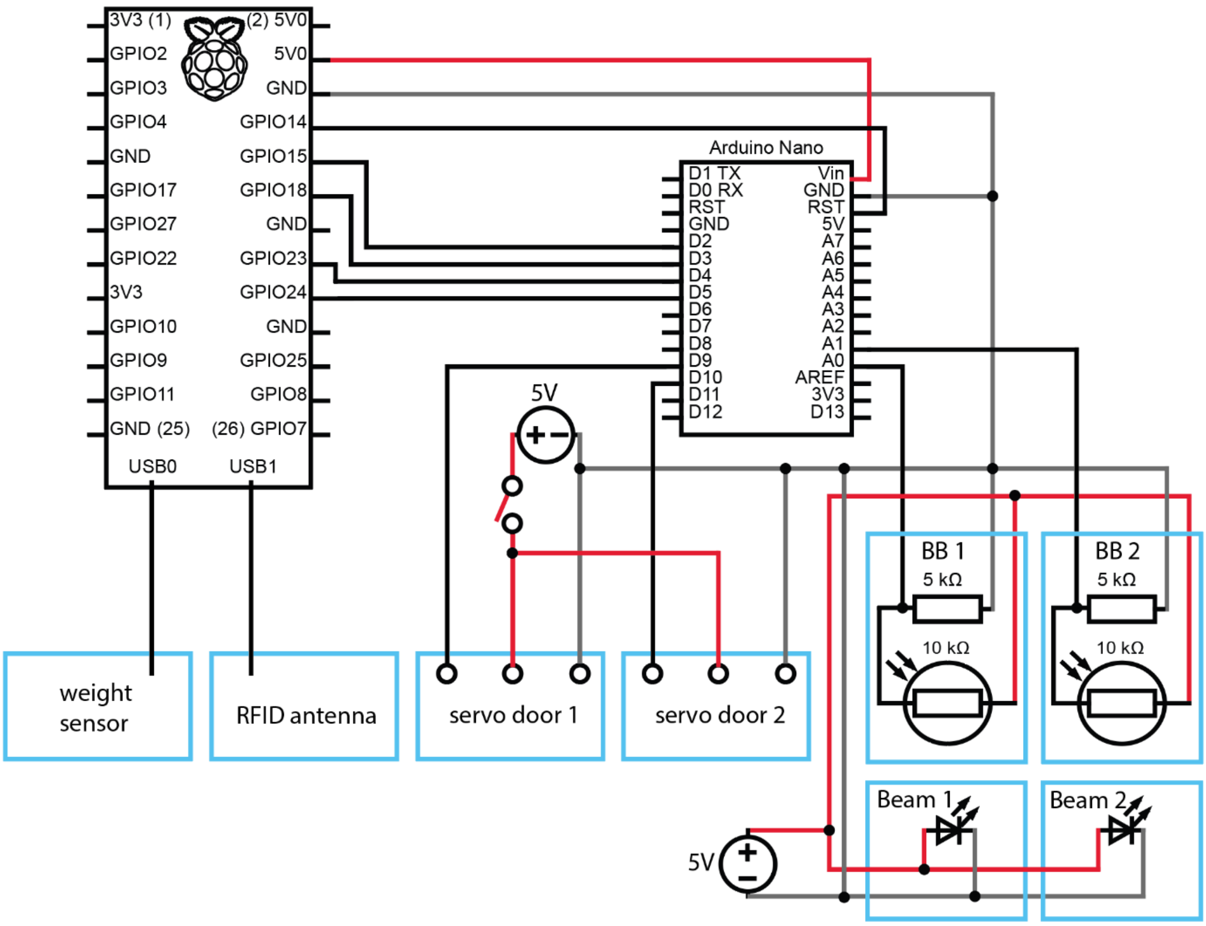

Figure 11. Wiring diagram.

Code and software set up

An Arduino board is used to detect beam breaks, reporting them to a Raspberry Pi, and to open/close doors when commanded by the Raspberry Pi. Software and code is set up following these steps:

1) On the Raspberry Pi (we have used models 400 and 4b) with the operating system installed (Raspberry Pi OS, released 21-02-2023), Arduino IDE (version 1.8.19) is used to install the Servo library (v 1.1.8).
2) The Arduino code *SEM_ard_nano.ino* is downloaded from the supporting files or [1] and flashed to the Arduino Nano. An Arduino Mega board can also be used.
3) For setting up Python (Python 3.9.2 is preinstalled on the Raspberry Pi OS), Pandas and Numpy libraries are first installed via the terminal (e.g. `sudo pip3 install numpy` and `sudo pip3 install pandas`). The *requirements.pip* file is downloaded from [2] or supporting files of this document. After navigating in terminal to the folder containing the *requirements.pip* file, all listed requirements are installed by typing `pip3 install -r requirements.pip`.
4) The helper script *SEM_functions.py* and the main script *SEM_only.py* are downloaded from [2] or supporting files of this document and placed in the same folder.
5) Normal operation of beam break sensors is tested empirically by uncommenting lines 44, 70-73 and 81-84 in the Arduino code and flashing the code to the board. It is best to test one sensor at a time and only uncomment the lines corresponding to BB1 or BB2. Then, enabling the serial monitor in Arduino IDE, values can be read out while occluding the sensor to

simulate animal detection. The values for an open beam should be approximately the same in darkness and with room lights on. If this is not the case, the 3D-printed part holding the sensors can be painted black with, e.g., black nail polish, or a piece of transparent red plastic can be placed in front of the detector. The value for an occluded beam should be less than half the open beam value. If this is not the case, beam targeting may be improved while monitoring the values. Beam detection thresholds are set on lines 87 and 96. After testing, lines 44, 70-73 and 81-84 need to be commented out again to operate the doors rapidly, and the code needs to be flashed onto the board.

6) On first installation, the scale needs to be calibrated and the correct settings must be set according to the manufacturer's instructions https://learn.sparkfun.com/tutorials/openscale-applications-and-hookup-guide?_ga=2.14390071.1017329722.1674997605-1611569064.1667393722.
   Briefly, after ensuring the correct USB port selection, a serial connection to the OpenScale is launched in Arduino IDE and the control menu is accessed by pressing 'x'. Baudrate is set to 9600, report rate to 120 ms, units to kg, decimals to 4, average amount to 1 and the serial trigger is switched 'off'. The scale is then tared to zero and calibrated with a 25 g weight. Calibration should be repeated daily in the beginning as the load cell can 'creep' somewhat after installation.
7) The correct position of the doors and appropriate door angles will likely vary with each build. The door angles are set by trial and error on lines 21-24 of the Arduino code. As a standard practice, the levers are installed such that they cannot hit the floor plate above them. For this, the servos are powered off, the servo hub is manually turned to the extreme position and the lever is installed such that it is near but not touching the floor plate. Thus, the default code has 'closed' values of 179 degrees and suitable 'open' values can be found by testing values less than 90 degrees. In case of collision, the servos should be powered down immediately and the code should be adjusted to values larger 'open' values (typically closer to 90). When powering up the system the servos can receive extreme commands. Therefore it is best to power up the servos last.
8) Two manual setup operations must be done every time after restarting the Pi:
   a. The PiGPIO daemon is launched from the terminal (`sudo pigpiod`).
   b. The servos are powered up and the door angles are checked/changed for safe operation (see section above).
9) Now the main script *SEM_only.py* can be run via the terminal or an IDE (we use Thonny Python IDE).

Adjustments and Troubleshooting

The user can change parameters, like how long the mouse must minimally spend on the other side before permitted through, heavy and light weight limits and where data is stored, in *SEM_functions.py*.

Should the code fail to open a connection to the scale or give an error that the connection is already in use, ensure that the Arduino IDE serial monitor is closed while running the code and that the baudrate set in the code matches the one in the serial connection to the OpenScale. In some builds it has been helpful to open and close a connection through Arduino IDE serial monitor and close Arduino IDE, after which the python code has been able to connect better.

The speed of door movements is set by the slowness constant on line 7 of the Arduino code. This value corresponds to the time, in microseconds, it takes to move the servo by 1 degree. We have found 8000 ideal.

To register new RFID tags, the main script can be used. When a new animal enters the SEM the new tag is printed into the terminal and can then be copied into *SEM_functions.py* as a known animal tag.

References

(1) *Switch_maze/Modules_SM/SingleEntryModule/SEM_ard_nano at main · MaheshKarnani/Switch_maze · GitHub*. https://github.com/MaheshKarnani/Switch_maze/tree/main/Modules_SM/SingleEntryModule/SEM_ard_nano (accessed 2023-06-26).

(2) *Switch_maze/Modules_SM/SingleEntryModule at main · MaheshKarnani/Switch_maze · GitHub*. https://github.com/MaheshKarnani/Switch_maze/tree/main/Modules_SM/SingleEntryModule (accessed 2023-06-26).