Build instructions for a long-term behavioural enclosure for measuring motivational switching in mice

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Abstract

Switching between motivated behaviours, such as feeding, drinking and socializing, is important for survival in a dynamic, social environment. Inflexible repetitive behaviours are a hallmark of many neuropsychiatric disorders. Studies of the neural mechanisms underlying motivated behaviours, i.e., drives, seldom focus on switching between them. This is partly due to a lack of appropriate behavioural measurement systems. In this study, we build and test a device for measuring motivational switching in mice, the Switchmaze. We present build instructions using affordable off-the-shelf components, and openly accessible acquisition and analysis scripts.

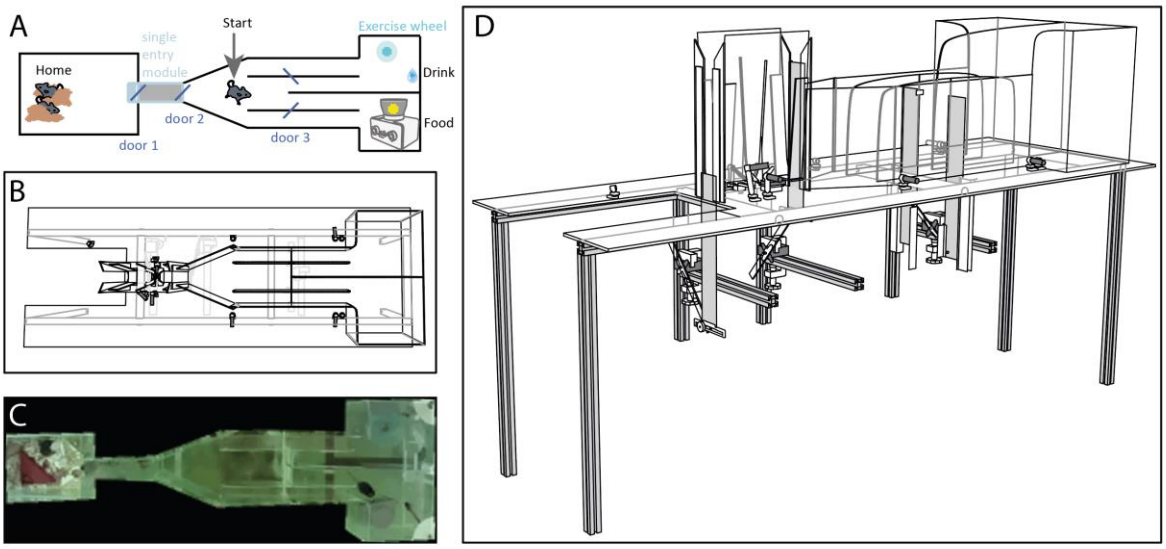

Figure 1, Switchmaze. A, Schematic; B, CAD model from above; C, picture from above; D, CAD model.

Introduction

Motivated behaviours such as feeding, drinking and social interaction form a scaffold upon which we build our daily lives. These are generated by neural mechanisms, here termed drives[1,2]. In health, these drives alternate to meet internal needs, while also being controlled by availability of goal objects[3] and controllable through conscious effort. Therefore, the neural mechanisms behind motivation selection are likely to be complicated and probabilistic, requiring measurements of repeatable discretized behavioural epochs. Here, we set up an apparatus (Figure 1) to measure motivational switching in mice on the timescale of milliseconds.

Rapid motivational switching is not studied often. Instead, typical assays measure *initiation* and overall duration of motivated behaviours, often indicated via operant levers/nose-pokes[4–7]. On a slower timescale, a probabilistic behavioural satiety sequence of feeding-grooming-resting, occurring over the course of an hour has been described in rodents and crayfish[8,9]. Transitions between sequential motivated behaviours in the home cage environment are seldom measured at a high temporal resolution[10–12]. This may be because motivational switching can be highly disordered in the home cage where the goal objects are constantly accessible. This makes quantification difficult and does not reflect typical conditions outside the laboratory. Therefore, we set up a naturalistic sequential foraging task which discretizes motivational switching.

The Switchmaze is an automated, liveable maze where feeding and drinking are discretized spatially. Previous automated measurement habitats have focused on recording/training for a head-fixed task[13–15] or tracking individualistic traits, like sociability, across a long time course[16–19]. Our goal was to capture spontaneous switching between motivations for feeding, drinking and returning to the nest for social interaction, enabling studies of the underlying neural mechanisms, i.e., drive switching. The Switchmaze consists of a home cage coupled through a single entry module to a foraging environment where an animal can serially retrieve a quantum of food or water from an isometrically placed decision point (Figure 1).

Materials

Table 1, Bill of materials for the Switchmaze. Main parts for the three modules with separate build instructions in references[20–22] are labelled SEM (single entry module), RW (running wheel) and SWD (sensing water dispenser).

Part	Count, vol. or length	Manufacturer	Manufacturer serial # or *.stl file	Approximate cost, EUR	Part	Module
Arduino Mega	1	Arduino	A000067	33.6		
Raspberry Pi 4b or 400	1	Raspberry Pi	KW-2646	45 – 95.0		
Servo motor	4	Master	1556176 - 62	112.0		SEM 2x, RW 1x
Small mirror tiles (1 x 1 cm), self-adhesive	2	e.g., amazon.com, or a piece of compact disc	N/A	10.0		
Switch	1	Adafruit	P3064	2.9		
5V power supply	2	RS Pro	124-2183	23.0		
wire	2 m	RS Pro	196-4225	0.8		
2-core shielded cable	20 m	Lapp	0034302	10.0		

3-core shielded cable	2 m	Lapp	1600207	6.0		SWD
4-core shielded cable	2 m	Lapp	0034304	1.0		RW
RFID tags	4	Sparkfun	SEN-09416	22.0		
RFID tag implantation syringe	1	DHgate	533480816	2.2		
10 kOhm resistor	6	TE Connectivity	LR1F10K	0.6		SEM 2x
Load cell 100 g	1	SparkFun	TAL221 SEN-14727	9.4		SEM
Open scale	1	SparkFun	SEN-13261	32.5		SEM
RFID antenna	1	SparkFun	ID-20LA SEN-11828	33.7		SEM
RFID USB reader	1	SparkFun	SEN-09963	26.3		SEM
Mini USB cable	2	SparkFun	CAB-13243	4.0		SEM
650nm Laser Diode	5	SparkFun	P1054	28.0		SEM 2x
Photoresistor	5	SparkFun	SEN-09088	4.0		SEM 2x
Rotary encoder	1	Nidec Copal Electronics	REC16B50-201	20.7		RW
Transparent film A4 pieces	2	Staedtler	636 10DT6F	0.4		SEM
‘flying saucer’ running wheel for small rodents	1	Ware pet products	#03281	7.0		RW
Rubber tubing 5mm ID/ 7mm OD	20 mm	RS Pro	235-4806	1.0		RW
Female to male jumper wire connectors	25	MikroElectronika	MIKROE-511	8.4		
FED3	1	https://github.com/KravitzLabDevices/FED3 (or bought prebuilt from Labmaker or OEPS)		200.0		
Bio-Serv™ Dustless Precision Pellets™ for Rodents	1	Bio-Serv™	F05684	162.0		
Relay module	1	TRU Components	TC-9927156	5.0		SWD
Varistor 25VAC	1	TDK	B72205S0250K101	0.26		SWD

Resistor 10MOhm	1	Vishay	594-CBB0207001003GCT	0.6		SWD
Power source 24V	1	RS-PRO	175-3306	10.0		SWD
Solenoid valve 24V	1	SMC	VDW12GA	17.2		SWD
50 ml syringe	1	Terumo	SS 50L1	5.4		SWD
2.5 ml syringe	1	Terumo	SS 02LE1	0.5		SWD
21 G hypodermic needle	2	Terumo	AN*2150R1	0.1		SWD
3 mm (ID)/ 5mm (OD) silicone tubing	100 mm	Saint Gobain	760210	1.5		SWD
M5 threaded nylon barbed tube fitting	2	McMaster-Carr	5463K557	1.2		SWD
Alligator clip	1	RS Pro	738-5856	2.8		SWD
Chloroform*	10 ml	Sigma-Aldrich	288306	5.0		
350 mm X 50 mm rectangles of 0.5 mm thick aluminium sheet	4	Reely	297895 - 8J	16.0	door	SEM 2x
3D-printed parts	3		bb345_send.stl	20.0 all 3D-printed parts	A	
	3		bb345_receive.stl		B	
	1		nest_ladder.stl		C	
	2		HSD_door_stopper.stl		D	
	1		base.stl			RW
	1		cover.stl			RW
	2		HSD_door_guide_top.stl		E	
	1		HSD_door_guide_bottomR.stl		F	
	1		HSD_door_guide_bottomL.stl		G	
	1		scale1.stl			SEM
	1		scale2.stl			SEM
	1		scale3.stl			SEM
	2		scale4.stl			SEM
	10		bb_base.stl		H	
	10		bb_base_nut.stl		I	
	1		bb1_send.stl			SEM

	1		bb1_receive.stl			SEM
	1		bb2_send.stl			SEM
	1		bb2_receive.stl			SEM
	2		door_base.stl			SEM
	2		door_base_nut.stl			SEM
	3		servo_clamp.stl		J	SEM 2x
	3		servo_bolt.stl		K	SEM 2x
	3		servo_bolt_cap.stl		L	SEM 2x
	1		rfid_base.stl			SEM
3 mm Acrylic sheet parts	1		middle_wall.svg	50.0 all acrylic parts	M	
	1		FED3_diagonal_wall.svg		N	
	1		FED3_base.svg		O	
	2		FED3_structural_support.svg		P	
	1		floor.svg		Q	
	1		HSD_door_lever.svg		R	
	4		inner_wall.svg		S	
	2		long_side_wall.svg		T	
	2		connecting_side_wall.svg		U	
	2		goal_wall1.svg		V	
	1		nest_back_wall.svg		W	
	1		nest_back_wall_water_base.svg		X	
	2		nest_back_wall_water_base_support.svg		Y	
	1		nest_front_wall_connector.svg		Z	
	2		nest_front_wall_half.svg		AA	
	2		nest_long_wall.svg		BB	
	2		goal_wall2_side.svg		CC	
	1		goal_wall3_back.svg		DD	
	4		door_guide_back.svg			SEM

	4		door_guide_spacer.svg			SEM
	4		sem_support.svg			SEM
	2		door_lever.svg			SEM
1 mm Acrylic sheet parts	4		door_guide_front.svg			SEM
	2		prevent_burrowing.svg			SEM
20 mm square profile aluminum rail, 500 mm length	4	McMaster-Carr	5537T101	132.2 all rail parts		
20 mm square profile aluminum rail, 300 mm length	9	McMaster-Carr				SEM 4x
20 mm square profile aluminum rail, 1400 mm length	2	McMaster-Carr				
M6 cap screws, 30 mm length	6	McMaster-Carr	90128A266	9.2		
M6 grub/set screws, 30 mm length	10	McMaster-Carr	92015A136	6.5		SEM 4x
M6 Drop-In T-Nut	10	Thorlabs	XE25T1/M	30.5		SEM 4x
M3 cap screws, 30 mm length	4	McMaster-Carr	91290A171	7.2		SEM 2x
M3 cap screws, 10 mm length	5	McMaster-Carr	91274A105	6.4		SEM 2x
M3 nuts	6	McMaster-Carr	90592A085	2.6		

Approx. total cost: 1137 – 1187 EUR

* Welding acrylic and 3D-printed PLA parts 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/Switchmaze

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

Build instructions

General description

The Switchmaze incorporates three modules explained in separate documents (single entry module[20], sensing water dispenser[22] and timed running wheel[21]), a FED3 pellet dispenser[23], a horizontally sliding door (HSD, described below) and a home cage with added walls (described below and in [20]). These parts are embedded in a simple maze made of 3 mm thick acrylic sheet built on an elevated 1400 by 500 mm floor plate (Figure 1D, interactive 3D model in Supporting Files).

Most parts are attached to each other using chemical welding with chloroform. This is a hazardous technique which requires adequate ventilation and PPE including gloves and an appropriate mask. Using minute quantities of chloroform minimizes risks, so it is strongly recommended to dispense chloroform sparingly from a 1 ml syringe (blunted needle for safety). The general idea is to firmly hold two pieces of acrylic sheet (or an acrylic sheet and 3D-printed PLA component) against each other and allow a drop of chloroform to seep in between the pieces and keep holding them in place for about one minute. After this, the bond will have formed and most of the free chloroform will have evaporated. If needed, the bond can usually be broken by bending the pieces, and remade, as long as the pieces have enough contact surface with each other. Acrylic cement (e.g., Acrifix 192) may be used to form permanent, cavity-free bonds after the initial weld.

Mechanical assembly

1. Frame and extended floor plate:

A support frame for the floor plate is built using three 300 mm, two 1400 mm and four 500 mm long 20 mm square profile aluminium rail according to Figure 2 and lay the 3mm acrylic sheet floor plate on top (part Q). A 6 mm drill piece suitable for aluminium is used 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. Drop-in nuts and grub screws are used for the 500 mm legs. A minimum height of 500 mm from is recommended as the doors need to operate some distance below the floor sheet.

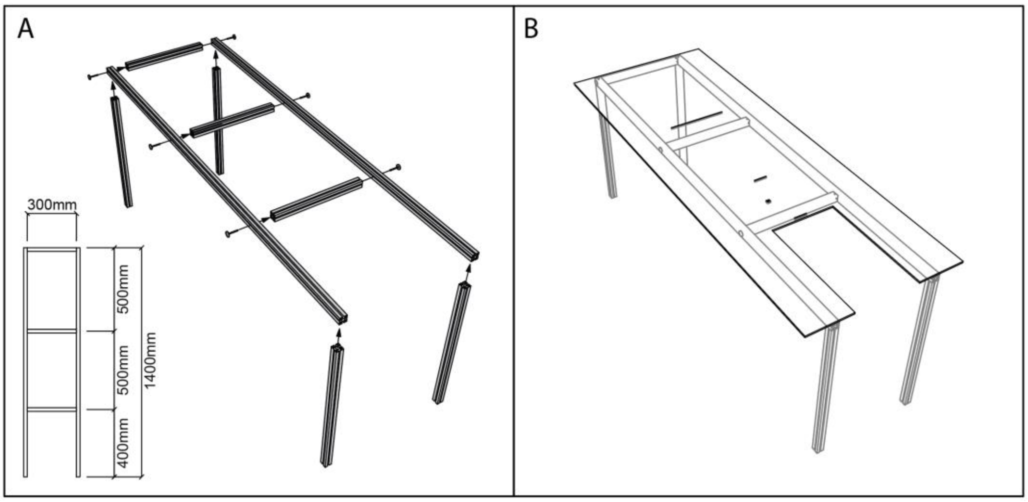

Figure 2, Aluminium rail frame without (A) and with floor plate (B).

2. *Single entry module:*

The SEM is assembled on the extended floor plate following previous instructions[20] (Figure 3), using parts labelled SEM in the 'Module' column of Table 1. Only the mechanical assembly is required as the electrical connections will be made differently from[20] on the same Arduino Mega board and Raspberry Pi controlling all aspects of the Switchmaze (see 'Electronics' section below).

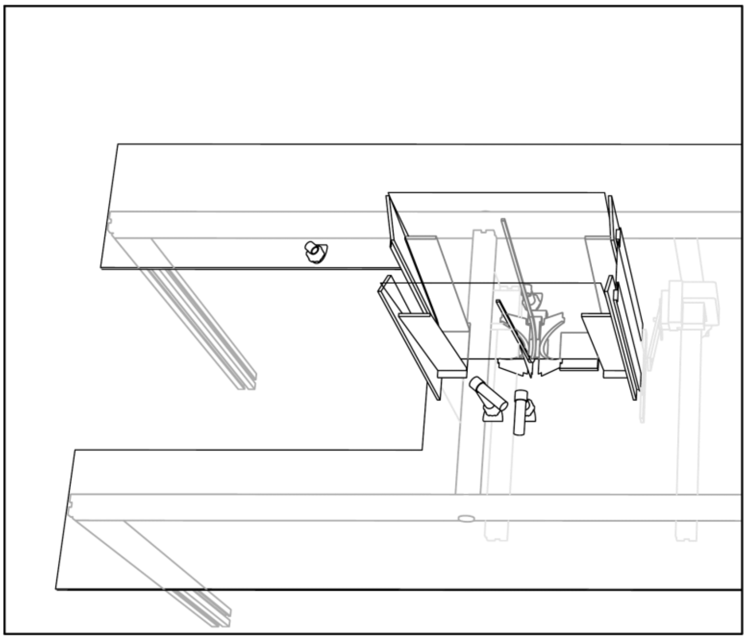

Figure 3, Single entry module.

3. *Walls of the foraging environment:*

Next, building the maze should be continued from the SEM toward the reward zone. All walls are installed by chemical welding using a few drops of chloroform and gently pushing the bottom edge of the wall against the floor plate for about one minute while the bond forms. Two heavy objects with a straight edge can useful, as the wall can be sandwiched in between them and allowed to bond with the floor for some minutes. It is best to use a ruler and a marker pen to draw in the wall positions first. After joining to the floor, each wall should be bonded to an adjacent wall, except for the four inner walls (see below). Walls should be joined in the following order:

A) First, the long side walls (part T, 400 x 200 mm) whose middles (400 mm long side) should align with the ends of the 2 x 200 mm HSD hole in the floor plate (Figure 4A).

B) Then the two connecting side walls (part U, 200 x 200 mm) should connect diagonally the long side walls and the SEM door frame (Figure 4B).

C) Then the five walls of the reward area, by sequentially attaching parts V (110 x 300 mm with one round corner), CC (200 x 300 mm) and DD (400 x 300 mm) to the ends of the long side walls (Figure 4C). The final positions of these pieces may need to be adjusted so it is best to use small amounts of chloroform and weld them initially in quick succession so they can be moved if necessary. When final positions are reached, a strong bond should be formed between adjacent walls and the floor using a few drops of chloroform and firm force on each joint.

D) The middle wall (part M, 400 x 300 mm with one round corner) is added to separate the two goal areas (Figure 4D).

E) The four inner walls (part S, 200 x 200 mm with one round corner) are added at a roughly 50 mm distance from the side walls to separate entry and exit passages to/from the goal areas (Figure 4E,F). Care must be taken to keep the inner walls away from the 2 x 200 mm HSD aperture (Figure 4G) as the HSD will slide horizontally through the gap between these walls.

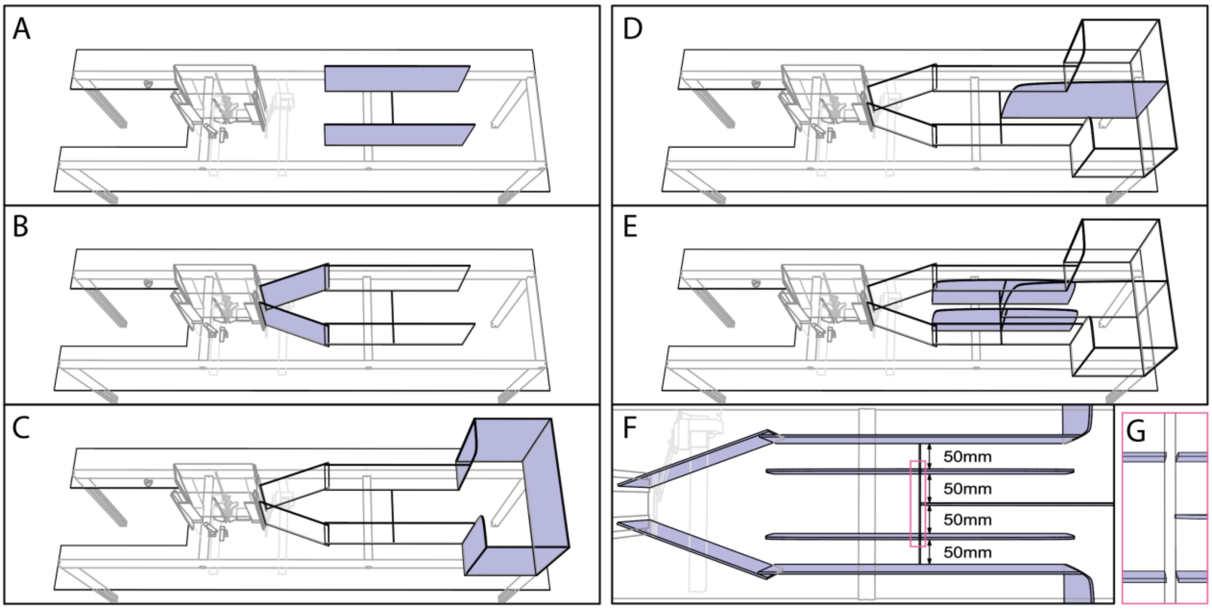

Figure 4, Walls of the foraging environment.

4. ***Horizontal Sliding Door:***

Using the drop-in T-nuts and M6 grub screws, a 300 mm aluminium rail is hung downward from the support frame, and another 300 mm aluminium rail is attached perpendicular to that (Figure 5A). A servo motor is attached on top of the perpendicular rail using a 3D-printed clamp (parts J-L; Figure 5B). The hub of the servo should be aligned with the middle wall (Figure 4D).

The HSD lever is glued to a servo hub attachment: The HSD lever made of 3 mm acrylic sheet (part R) is attached at its middle hole to a servo hub attachment with a flat surface (provided with the servo) using a 10 mm M3 screw. Once tightened, working in a well-ventilated space/chemical hood, 1-2 drops of chloroform are added on the joint to activate the surfaces. After a few minutes 2-3 drops of superglue are added to the joint. After the glue has set, the M3 screw can be removed. Epoxy glue is used to reinforce the joint as it will need to withstand impacts in routine operation. The adhesives should be allowed to set completely such that the lever and hub attachment are held together strongly.

3 mm holes are drilled through the aluminium doors (part 'door'; 50 x 350 mm aluminium sheet) at the indicated locations (Figure 5C). Then, the 3D-printed bottom side door guides (part F and G) are

attached by sliding them on the edges of the doors as indicated and gluing in place with small drops of superglue (Figure 5C). These door guides will oppose each other when the door is in the closed mode (middle passage closed).

The doors can then be assembled by fastening the lever to the servo hub, making sure that the travel range is appropriate for door operation (the servo should be at its end of range when the lever is nearly touching the floor plate at near vertical angle), and attaching the doors with 10 mm M3 cap screws and nuts to the ends of the lever (Figure 5E). These screws should not be tightened all the way as the angle between the lever and doors changes during operation.

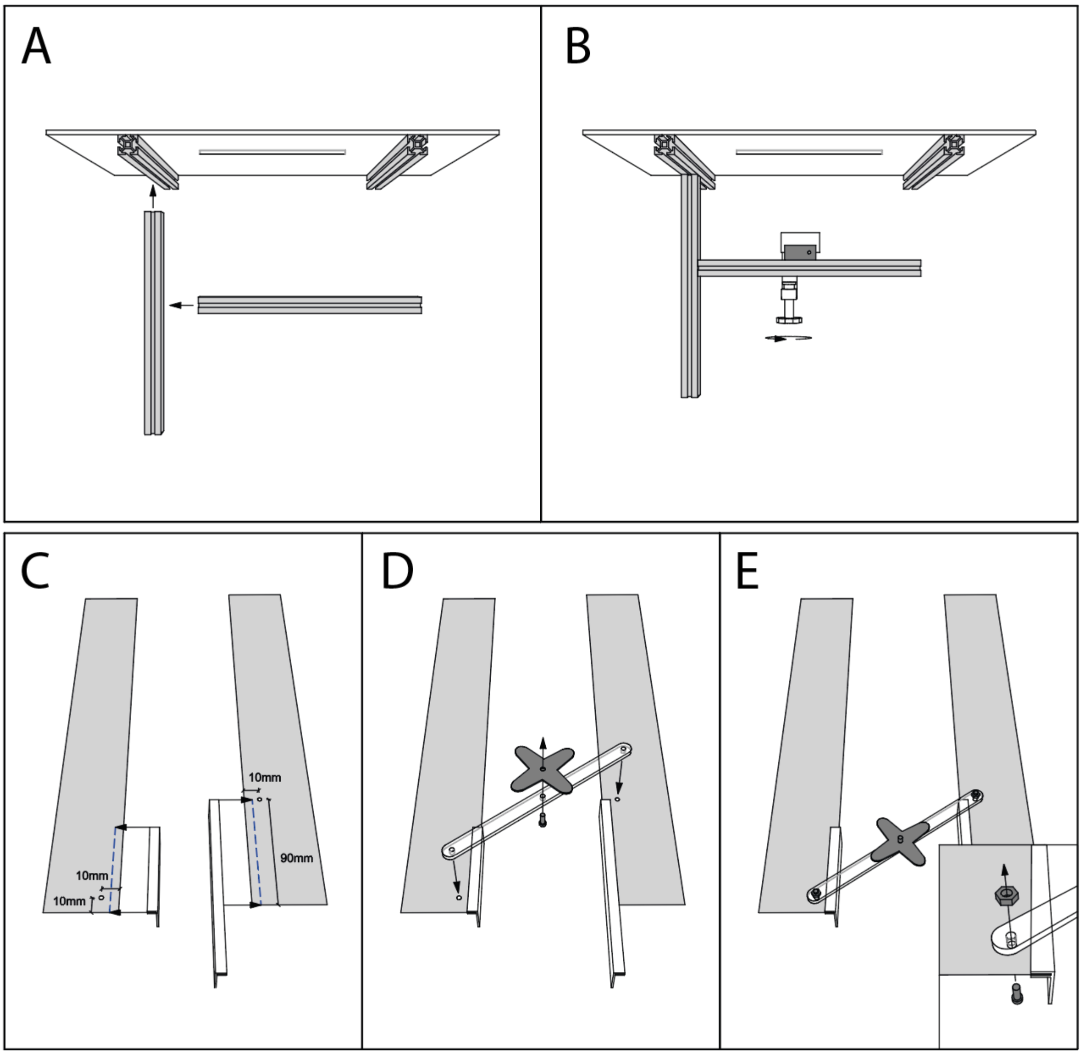

Figure 5, Assembling components of the HSD.

Finally, the limiting stoppers and top guide piece are attached to the doors (Figure 6A). The top door guide (part E) is glued to the top of one door (Figure 6B), such that it will oppose the middle wall when the door is closed. To fit the limiting door stoppers (part D), the doors must first be moved to the fully open position (Figure 6A). This can be done manually as long as the servo is not powered. The door stoppers should be welded to the floor plate with a small amount of chloroform such that they touch the doors when they are in the open position, forcing them to be vertical (Figure 6C,D). These stoppers function to keep the doors vertical when they are in the open position (middle passage open), and therefore should make contact with the aluminium doors in the open position (Figure 6D). The top and bottom door guides function to keep the doors vertical in the closed position (Figure 6E).

When the door is in operation, the angle commands should be selected carefully so that the stoppers and guides keep the doors vertical but no force is left acting on the doors at open or closed angle, as this will damage the components. I.e., the servo should have free range to move a couple of degrees beyond the open position. Similarly, in the closed position, the doors should not generate a static force against each other, but should just right each other.

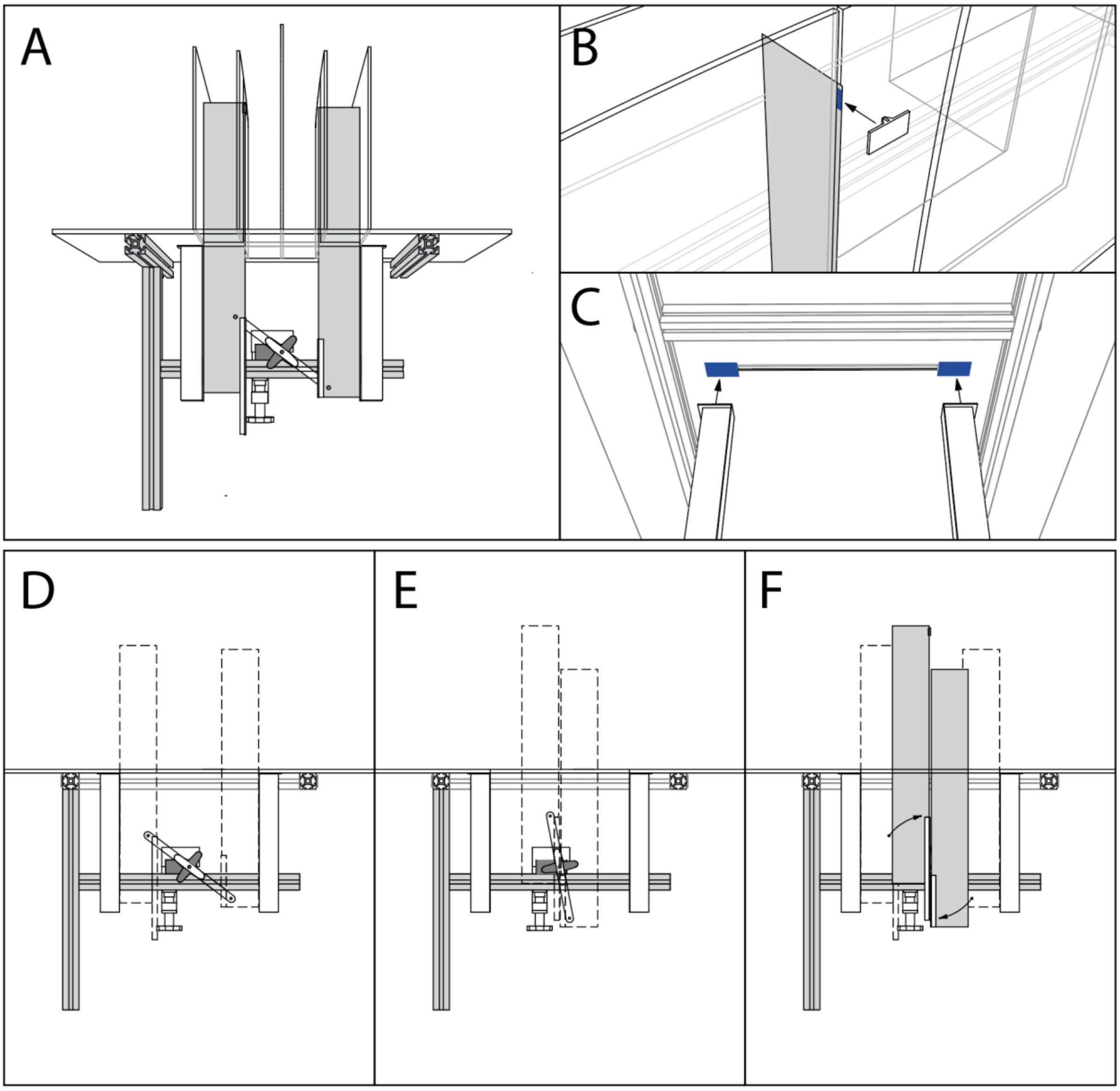

Figure 6, Horizontal sliding door with stoppers and guides.

5. ***Beam break detectors***:

Three individual beam break detectors are built from identical components using 3D-printed parts A,B,H and I (Figure 7A), diode lasers (Sparkfun P1054) and photoresistors wired to resistors. The laser and detector holders are built first (Figure 7A). Each has three parts: base (part H), tightening nut (part I) and a part with a ball joint (part A for the laser and part B for receiver). 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 7B) and a thin mirror, such as a piece of a compact disc is glued or welded to the middle wall so beams from senders 3 and 4 will be reflected to their detectors. Beams should always be about 10-15 mm from the floor to ensure animal detection. Beam targeting is checked by sighting through the senders to the detectors. A small drop of chloroform is used to weakly bond each holder to the floor plate, so they can be detached if necessary. Then the lasers and photoresistors are inserted into their holders (use small abount of glue if needed). The cylindrical part of the receivers should be printed using black plastic or coated with a black paint (e.g., black nail polish) or black tape so that the major source of detected light is the sender. Later when the lasers are operational, locations can be confirmed and the holders' base parts should be joined strongly to the floor plate with additional drops of chloroform. The ball joints allow fine tuning the beam targeting.

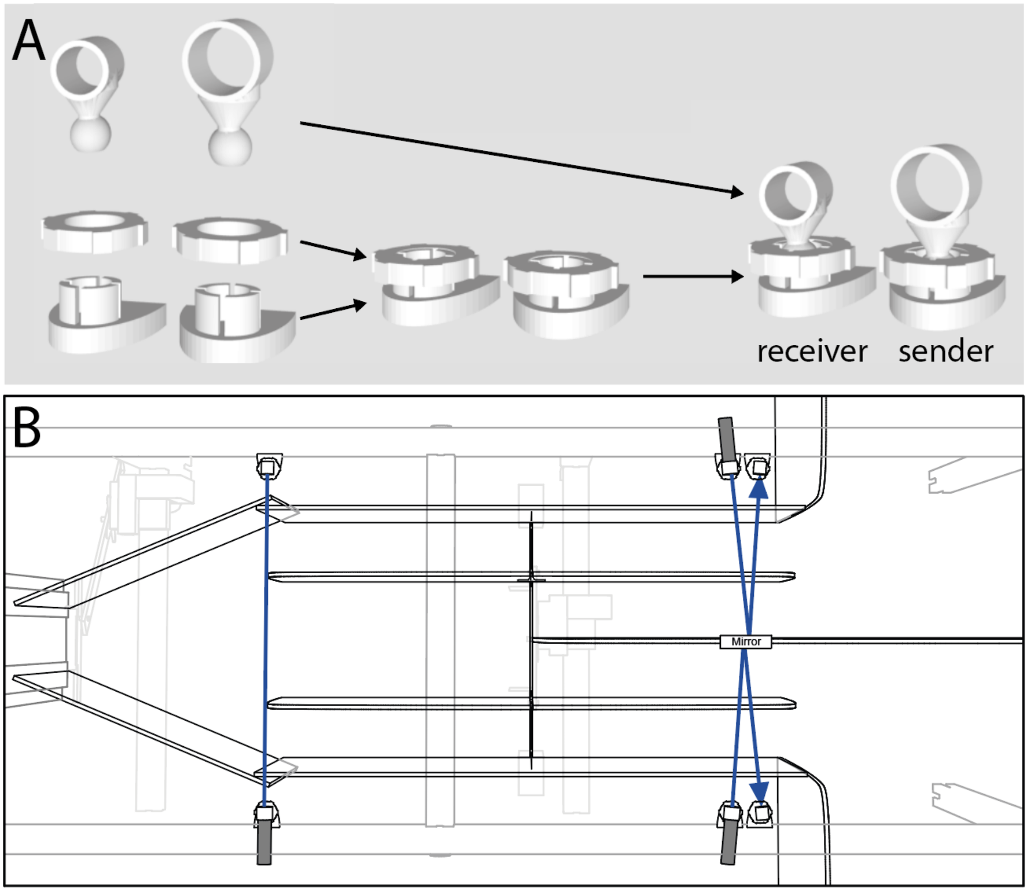

Figure 7, Beam break detectors. A, Each sender and receiver unit is assembled from three 3D-printed parts. B, Beam break detectors are placed at a safe distance (>100 mm) from door 3. For detectors 4 and 5, a thin mirror, such as a piece of a compact disc, is glued to the middle wall.

At this stage the device should look like Figure 1B and D.

6. Home cage:

The home cage bottom of each cohort of mice is used as the home area in the Switchmaze (Figure 8A)[20]. It has a top edge protrusion 'lip' (Figure 8B) which allows it to slide into the 200 mm wide slot on the floor plate.

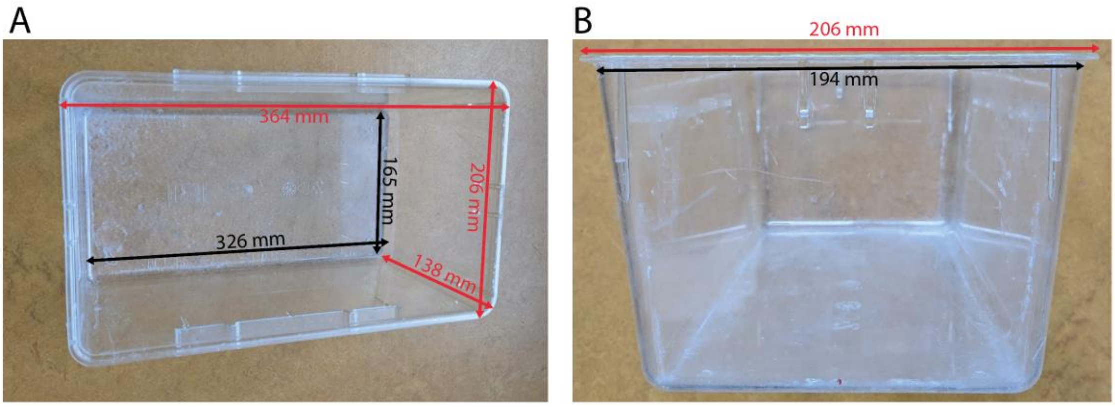

Figure 8, Home cage bottom.

A wall unit for the home cage is built from 3 mm thick acrylic sheet and a 3D-printed ladder (parts C, W, X, Y, Z, AA, BB). These are welded together with chloroform sequentially as shown in Figure 9.

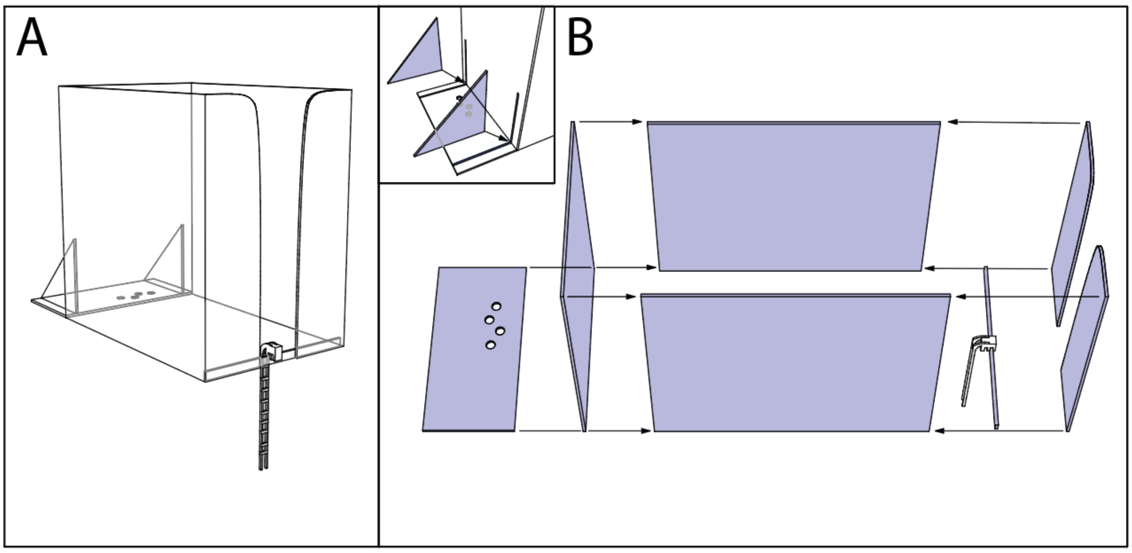

Figure 9, Home wall assembly.

7. *Goal modules:*

Food, water and a running wheel are provided as goal modules that can be easily swapped between the goal areas, removed or replaced with other items, such as social chambers or novel objects.

a. A sensing water dispenser (SWD) is built following previous instructions[22], using parts labelled SWD in the 'Module' column of Table 1. Only mechanical assembly is required as the electrical connections will be made differently from[22] on the same Arduino Mega board and Raspberry Pi controlling all aspects of the Switchmaze (see 'Electronics' section below). The water spout is introduced through a 4 mm diameter hole drilled in the wall at about 10 mm above the floor plate (Figure 10A).
b. A Feeding Experimentation Device, FED3[23] is built following Kravitz lab instructions https://github.com/KravitzLabDevices/FED3 (or bought prebuilt) and modified for simultaneous input and output:
   i. A 3-core cable is soldered on the FED3 main PCB front side such that it carries the BNC output signal lead, ground and Feather M0 Adalogger pin 9.
   ii. The FED3.cpp code (December 2020 version) is modified by changing line 352 from `pinMode(BNC_OUT, INPUT_PULLDOWN);` to `pinMode(BNC_IN, INPUT_PULLDOWN);` and line 354 from `if (digitalRead(BNC_OUT) == HIGH){` to `if (digitalRead(BNC_IN) == HIGH){`.
   iii. The FED3.h code (December 2020 version) is modified by adding `#define BNC_IN                    9` between lines 58 and 59.
   iv. After these changes, the 'Dispenser' code is flashed to the FED3.

   With these modifications, the FED3 will dispense a pellet when the Feather M0 Adalogger pin 9 goes high and will report a pellet retrieval on the usual output BNC port (A0).

   The FED3 is placed in a diagonal wall assembly that is chemically welded together using parts N and O and two copies of part P (Figure 10B-D). This structure makes it easy to take out the FED3 for cleaning and protects its cables.
c. A timed running wheel is built following previous instructions[21], using parts labelled RW in the 'Module' column of Table 1. Only mechanical assembly is required as the electrical connections will be made differently from[21] on the same Arduino Mega board and Raspberry Pi controlling all aspects of the Switchmaze (see 'Electronics' section below). A 10 mm hole is drilled in the floor plate to pass the running wheel cable. However, given the low use of the running wheel in our results (see below), it can likely be left out of the build.

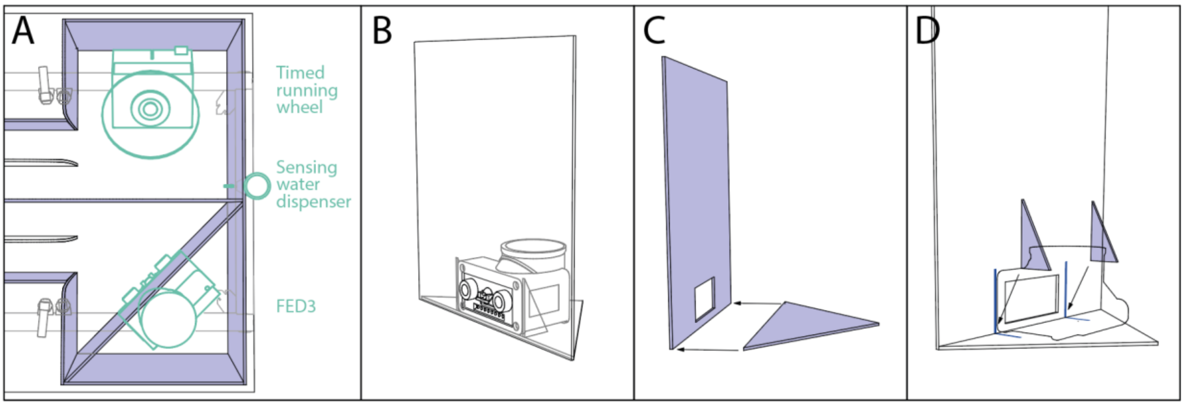

Figure 10, goal modules. A, placement of goal modules (cyan). B-D, FED3 module assembly.

Electronics

See Figure 11 for connections. 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 Mega through 12 digital lines. Additionally, the Arduino connects to the capacitive lick detector (input), visual output of detected licks (LED output, line D32), the servo motors (output) and the beam break sensors (input). The Raspberry Pi controls the Arduino and, directly, pellet dispensing from the FED3 (output) and water dispensing (output). Additionally the Raspberry Pi receives beam break and lick data from the Arduino, and, directly, pellet retrieval from the FED3 (input) and wheel rotation data (input).

Two-core shielded cable is used to connect servo motors, beam break detectors and FED3, and 4-core shielded cable for the timed running wheel and dispensing lick sensor. A switch (Adafruit P3064) is necessary on the servo power source, as the motors can damage components if powered up during start-up. This also makes troubleshooting easier as the motors can rapidly be turned off when needed and levers returned manually to safe operating range.

The 650nm Laser Diodes (SparkFun P1054) are connected to a separate 5V DC source (Adafruit P3064) as are beams and beam break sensors, which connect to the Arduino across 10 kOhm resistors. Importantly, all grounds should be wired together, apart from the 24V solenoid valve power source which is on a separate circuit.

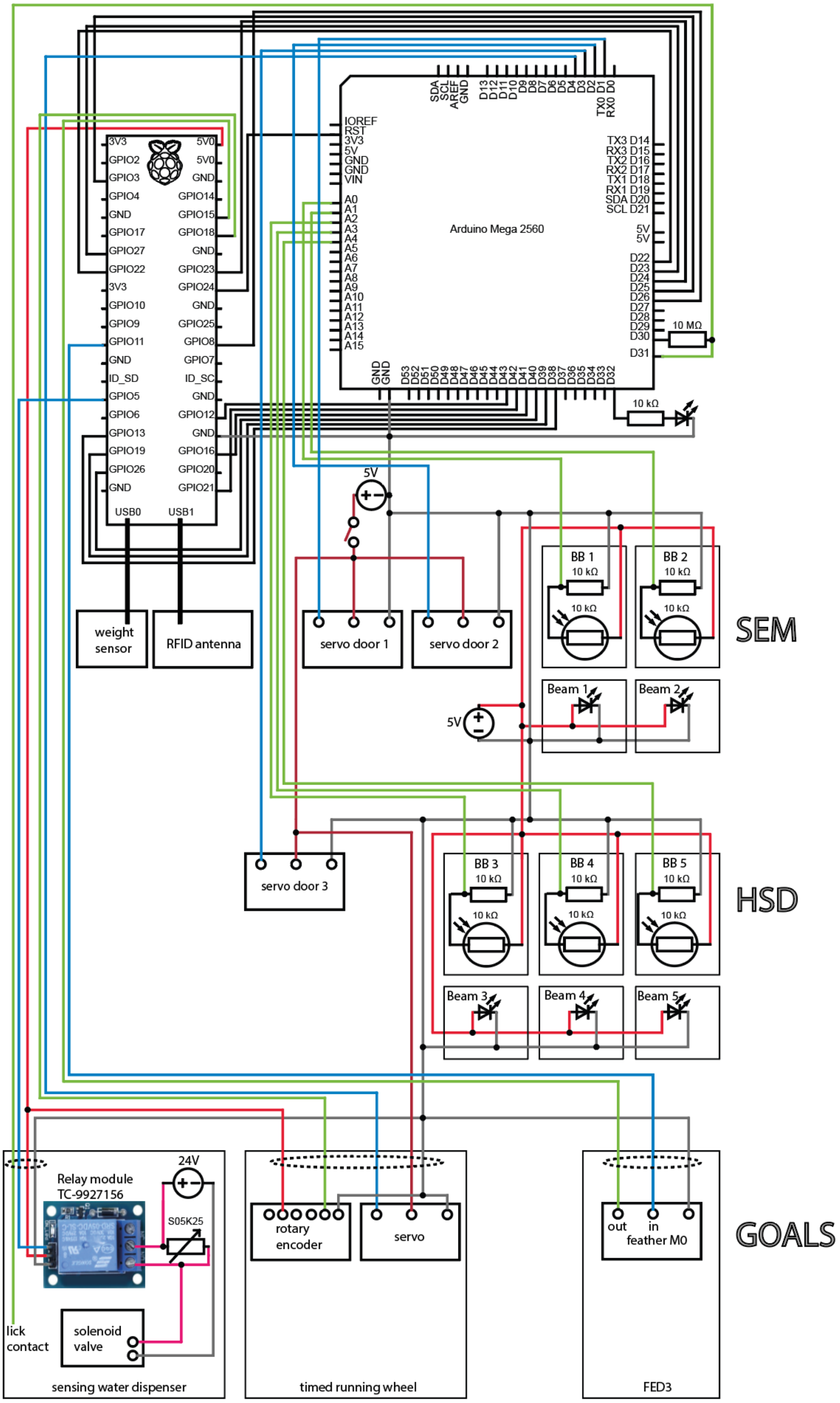

Figure 11, Wiring diagram. Red = positive voltage source, green = sensor data, blue = output commands.

Code and software set up

An Arduino Mega is set up for moving the servos, sensing the beam detectors and capacitive sensing of the lick spout. A Raspberry Pi is set up to control the experiment and log data. One can set up the software and code 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 CapacitiveSensor[24] and Servo[25] libraries.

2. The Arduino code *Switch_maze_arduino_mega.ino* is downloaded from the supporting files or[26] and flashed to the Arduino Mega.

3. Normal operation of beam break sensors is tested empirically by uncommenting lines 173-182 (or sequentially each pair of lines to follow one BB at a time) in the Arduino code and flashing the code to the board. 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 186, 195, 204, 213 and 222. After testing, lines 173-182 need to be commented out again to operate doors rapidly, and code flashed onto the board. For further adjustments and troubleshooting see below.

4. 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[26] 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`.

5. All **.py* scripts and helper scripts should be downloaded from the supporting files or[26] and placed in the same folder.

6. On first installation, the scale needs to be calibrated and the correct settings must be set according to the manufacturer's instructions[28] (briefly outlined in[20]).

7. The angles that a servo needs to achieve to close and open a door will likely vary based on the exact distances used in each build, and are set by trial and error on lines 35-40 of the Arduino code. As a standard practice, install the levers such that they cannot hit the floor board above them, i.e., power off the servo, turn the servo hub to the extreme position and install the lever there such that it is near but not touching the floor. Because we do this, the default code has 'closed' values of 179 degrees. After that it will be simple to find a suitable 'open' value by testing values less than 90 degrees. In case the door lever collides with something, turn the power to the servos off rapidly from their power switch, go back to the code and bring the degree value 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:

1. The PiGPIO daemon is launched from the terminal (`sudo pigpiod`).
2. The servos are powered up and door angles are checked/changed for safe operation (see section above).

9. Now the main script can be run via the terminal or an IDE (we use Thonny Python IDE).

Adjustments and troubleshooting

Beam targeting

Beam break devices in the SEM are targeted as explained in [20], briefly: Beam break 1 is used for safely closing door 1: Beam 1 is targeted above door 1 such that when the door is open the beam hits the photoresistor. When the door is closed or an animal is on top of the open door, the beam is broken. Beam break 2 (BB2) is used for detecting an exiting animal at a safe distance from door 2 (>100 mm): Beam 2 is targeted through the sides of the single entry module such that an animal in the middle of the module will break it.

Beam break devices in the HSD (BB3-5) are used like BB2 to detect an animal when the beam is broken. These enable safe operation of the HSD and logging entries to the goals or start point. These beams should be at a height of approximately 15 mm above the floor.

Beam detector readings can be monitored in Arduino IDE serial monitor after uncommenting lines 173-182 in the Arduino code (or to see only the initial calibration readings, lines 146-155). After diagnostics, these lines need to be commented out again to operate doors rapidly. Beam detection thresholds are set on lines 186, 195, 204, 213 and 222.

Weight sensor parameter selection and calibration

The weight sensor is set up and calibrated according to the manufacturer's instructions[28]. 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.

Door speed

The speed of door movements is set by the slowness constant on line 13 of the Arduino code. This value corresponds to the time, in microseconds, it takes to move the servo by 1 degree. We have found 150 ideal.

Using the Switchmaze

Radio-frequency identification (RFID) tag implantation

20 wild-type C57BL6 male mice were used in this study. All experimental procedures were approved by the Netherlands Central Committee for Animal Experiments and the Animal Ethical Care Committee of the Vrije Universiteit Amsterdam (AVD11200202114477). To implant a glass RFID tag capsule under the skin, each mouse was anesthetized with sleep mix (i.p., fentanyl 0.05 mg/kg, medetomidine 0.5 mg/kg and midazolam 5 mg/kg in saline). An RFID chip (Sparkfun SEN-09416) was implanted under the chest skin using a non-medical ID transponder syringe RFID injector (e.g., DHgate 533480816). The wound was closed with tissue glue, anaesthesia was antagonized with wake mix (i.p., flumazenil 0.1 mg/ml and atipamezole 5 mg/ml in saline) and the animal received 0.05 mg/ml carprofen in drinking water for 2-4 days as post-operative pain medication.

Test measurement of discretized motivational transitions

To test the Switchmaze, mice were housed in the apparatus for up to a month in cohorts of 2-4 animals. RFID tagged animal tags were first read in by allowing animals to explore the open SEM while running the script *RFIDreader_newcohort_main.py*. After starting the script, RFID tagged animals were placed in the Switchmaze in their home cage and the nest wall assembly was put in place. After the RFID tags of all animals were detected (10 - 60 min of free exploration typically), the tags were written into lines 27-30 of *Switch_maze_functions.py* and normal operation was started by running *Switch_maze_main.py*. For the first two days, water was available *ad libitum* in the home cage and 2g/animal of dry chow was provided on the home cage floor. This was done to ensure adequate food and water intake during the time when entering the foraging environment is a new action. Therefore, mice would first enter the foraging environment due to exploration. In 6-48 h, they learned to use the goal objects and obtain food and water from the maze.

During the initial habituation period, there were very infrequent events where an animal got stuck in the foraging environment after a maze exit was triggered. This was likely due to the animal making an elongated probing posture while gripping a closing door with its hindleg. These situations were noticed by monitoring via an overhead camera and the incoming event data, and resolved by running the script *rescue_main.py* which stops maze operation, waits for a complete exit and reports it via email to the user.

During baseline operation, an animal consumed on average 189 ± 49 food pellets (14 mg) in a 24 h period, which corresponds to the typical number of small meals wild mice are estimated to eat in a night[29]. Upon most entries into the food pod, a pellet was consumed (93.9 ± 10.1 % of entries). 210 ± 130 water drops (10 μl) were retrieved and drinking occurred upon 98.5 ± 2.3 % of entries into the water pod. In a 24 h period, an animal ran on the running wheel on average 6.7 ± 13.3 revolutions during 5 ± 7 entries into the water pod which accounted for 4.0 ± 6.5 % of the entries. Therefore, despite the appeal of running wheels even to wild mice[30], use of the running wheel was marginal and is unlikely to affect the results. Food (201 ± 49) and water (212 ± 130) pod entries were approximately balanced over a 24 h period.

Animals exhibited stereotyped entries into the foraging environment (Figure 12A) to eat and drink, followed by exit back into the home cage, which we termed blocks (Figure 12B,C). Blocks lasted on average 6.3 ± 8.0 min and tended to occur in clusters across the group, such that the animals spent time together in the home cage (Figure 12A). This suggests that the animals returned to the home cage due to a strong social motivation. They would rarely stay in the foraging environment for longer than 17 minutes (Figure 12D; 95^{th} percentile = 16.9 min).

During each block, an animal typically made multiple entries to the food and drink areas (Figure 12A), termed trials (Figure 12E,F). Trials lasted on average 26.3 ± 34.1 s (measured from start position to return to start position, i.e., a full behavioural cycle through one goal area), and rarely more than 71 s (Figure 12G; 95^{th} percentile = 70.7 s). These short cycle durations (Figure 12F,G), together with the brief blocks, suggest that the foraging behaviour was economical and purposeful.

The recorded behavioural sequences allowed analysing motivation transition likelihoods[10,11] (Figure 12H). As expected from the efficient organization of a small number of multi-trial blocks per hour, transition types within the foraging environment were most frequent (food-to-food, food-to-drink, drink-to-drink and drink-to-food). Unexpectedly, blocks tended to begin with a food trial and end with a drink trial, which was reflected in more home-to-food transitions than home-to-drink and more drink-to-home transitions than food-to-home. These patterns were consistent across animals (Figure 12I).

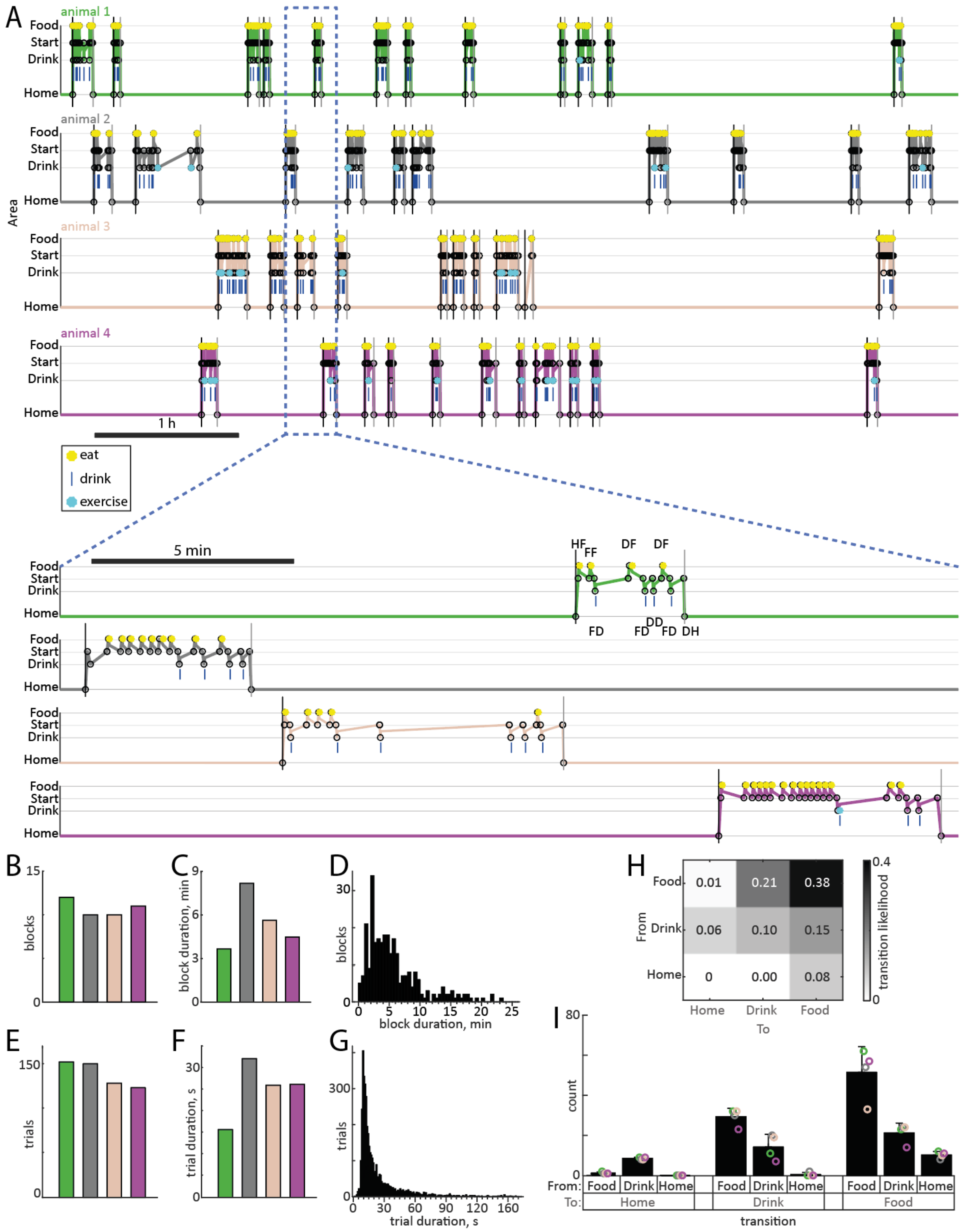

Figure 12, Example data. A, Ethograms of four mice entering the foraging environment one at a time for an open-ended block. One block for each animal shown in detail in the expanded time window (dashed box). Motivational transition types labelled for animal 1 (e.g., HF, home-to-food transition; H, home; F, food; D, drink). B,C,E,F, basic metrics for the 6 h session shown in A, colour coded by mouse. B, number of blocks; C, block duration; D, block durations across 20 animals in a 24 h period. E, number *of trials; F, trial duration; G, trial durations across 20 animals in a 24 h period. H, Mean transition likelihood matrix for the four animals in A. I, Raw number of transitions by type (from-to) and by animal (colour coded).*

Discussion

The Switchmaze is a low cost, open source and ethologically relevant semi-natural setting. The modular design allows for changing and modifying the affordances to enable various experiments, and the simple construction techniques in these instructions can be used readily to create diverse goal modules. Mice can stay in the apparatus indefinitely, as all the affordances they have in typical home cages are provided, and the maze is a highly enriched environment. Liveable experimental environments, like the Switchmaze, are ideal for probing internal models[31] and schemas[32] used in daily life. This is because the daily survival behaviours are discretized and repeated in a standard way every time they are expressed, in contrast to typical behavioural experiments where the animal is tested for minutes to hours in a small enclosure, followed by return to a different home cage environment. In addition to the automated measurement of movement, food and water intake of each mouse, the Switchmaze includes automated weighing, so daily welfare checks can be less intrusive and more targeted to individual mice. The Switchmaze is highly useful as it can be used to investigate motivation and drive switching (see the accompanying preprint[33]), in addition to measuring motivational transitions.

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References

1. Pfaff, D. W. *Drive: Neurobiological and Molecular Mechanisms of Sexual Motivation*. (MIT Press, 1999).
2. Berridge, K. C. Motivation concepts in behavioral neuroscience. *Physiology & Behavior* **81**, 179–209 (2004).
3. Ghoniem, A., van Dillen, L. F. & Hofmann, W. Choice architecture meets motivation science: How stimulus availability interacts with internal factors in shaping the desire for food. *Appetite* **155**, 104815 (2020).
4. Caprioli, D., Zeric, T., Thorndike, E. B. & Venniro, M. Persistent palatable food preference in rats with a history of limited and extended access to methamphetamine self-administration. *Addict Biol* **20**, 913–926 (2015).
5. Venniro, M. *et al.* Volitional social interaction prevents drug addiction in rat models. *Nat Neurosci* **21**, 1520–1529 (2018).

6. Reppucci, C. J. & Veenema, A. H. The social versus food preference test: A behavioral paradigm for studying competing motivated behaviors in rodents. *MethodsX* **7**, 101119 (2020).
7. Eiselt, A.-K. *et al.* Hunger or thirst state uncertainty is resolved by outcome evaluation in medial prefrontal cortex to guide decision-making. *Nat Neurosci* **24**, 907–912 (2021).
8. Rodgers, R. J., Holch, P. & Tallett, A. J. Behavioural satiety sequence (BSS): separating wheat from chaff in the behavioural pharmacology of appetite. *Pharmacol Biochem Behav* **97**, 3–14 (2010).
9. Tierney, A. J., MacKillop, I., Rosenbloom, T. & Werner, A. Post-feeding behavior in crayfish (Procambarus clarkii): Description of an invertebrate behavioral satiety sequence. *Physiology & Behavior* **213**, 112720 (2020).
10. Wee, R. W. S., Mishchanchuk, K., AlSubaie, R. & MacAskill, A. F. Internal state dependent control of feeding behaviour via hippocampal ghrelin signalling. 2021.11.05.467326 Preprint at https://doi.org/10.1101/2021.11.05.467326 (2022).
11. Burnett, C. J. *et al.* Need-based prioritization of behavior. *Elife* **8**, e44527 (2019).
12. Sotelo, M. I. *et al.* Lateral hypothalamic neuronal ensembles regulate pre-sleep nest-building behavior. *Current Biology* **32**, 806-822.e7 (2022).
13. Silasi, G. *et al.* Individualized tracking of self-directed motor learning in group-housed mice performing a skilled lever positioning task in the home cage. *J Neurophysiol* **119**, 337–346 (2018).
14. Woodard, C. L. *et al.* An Automated Home-Cage System to Assess Learning and Performance of a Skilled Motor Task in a Mouse Model of Huntington's Disease. *eNeuro* **4**, ENEURO.0141-17.2017 (2017).
15. Erskine, A., Bus, T., Herb, J. T. & Schaefer, A. T. AutonoMouse: High throughput operant conditioning reveals progressive impairment with graded olfactory bulb lesions. *PLoS ONE* **14**, e0211571 (2019).
16. Torquet, N. *et al.* Social interactions impact on the dopaminergic system and drive individuality. *Nat Commun* **9**, 3081 (2018).
17. Puścian, A. *et al.* Eco-HAB as a fully automated and ecologically relevant assessment of social impairments in mouse models of autism. *Elife* **5**, e19532 (2016).
18. Kiryk, A. *et al.* IntelliCage as a tool for measuring mouse behavior - 20 years perspective. *Behav Brain Res* **388**, 112620 (2020).
19. Kempermann, G. *et al.* The individuality paradigm: Automated longitudinal activity tracking of large cohorts of genetically identical mice in an enriched environment. *Neurobiology of Disease* **175**, 105916 (2022).
20. Hartmann, C., Thönnes, Y. & Karnani, M. Single entry module for rodent work using Arduino and Raspberry Pi. *ResearchEquals* (2023) doi:10.53962/ryj7-rtqw.
21. Hartmann, C., Thönnes, Y. & Karnani, M. Timed running wheel for rodent work using Arduino and Raspberry Pi. *ResearchEquals* (2023) doi:10.53962/j5fx-4zy8.
22. Karnani, M. & Hartmann, C. Sensing water dispenser for rodent work using Arduino and Raspberry Pi. *ResearchEquals* (2023) doi:10.53962/g2r8-ysds.
23. Matikainen-Ankney, B. A. *et al.* An open-source device for measuring food intake and operant behavior in rodent home-cages. *Elife* **10**, e66173 (2021).
24. Stoffregen, P. *CapacitiveSensor Library*. (2023).
25. Servo - Arduino Reference. https://www.arduino.cc/reference/en/libraries/servo/.
26. Switch_maze/Modules_SM/SwitchMaze at main · MaheshKarnani/Switch_maze · GitHub. https://github.com/MaheshKarnani/Switch_maze/tree/main/Modules_SM/SwitchMaze.
27. MaheshKarnani. Switch_maze/Modules_SM. (2023).

28. OpenScale Applications and Hookup Guide - SparkFun Learn. https://learn.sparkfun.com/tutorials/openscale-applications-and-hookup-guide?_ga=2.14390071.1017329722.1674997605-1611569064.1667393722.
29. Latham, N. & Mason, G. From house mouse to mouse house: the behavioural biology of free-living Mus musculus and its implications in the laboratory. *Applied Animal Behaviour Science* **86**, 261–289 (2004).
30. Meijer, J. H. & Robbers, Y. Wheel running in the wild. *Proceedings of the Royal Society B: Biological Sciences* **281**, 20140210 (2014).
31. Keller, G. B. & Mrsic-Flogel, T. D. Predictive Processing: A Canonical Cortical Computation. *Neuron* **100**, 424–435 (2018).
32. Takeuchi, T. *et al.* Brain region networks for the assimilation of new associative memory into a schema. *Mol Brain* **15**, 24 (2022).
33. Hartmann, C., Mahajan, A., Razenberg, L. & Karnani, M. M. Switchmaze: automated, ongoing measurement of motivation and drive switching in mice. *ResearchEquals* (2023) doi:10.53962/ywda-9ghy.
34. Nguyen, K. P., O'Neal, T. J., Bolonduro, O. A., White, E. & Kravitz, A. V. Feeding Experimentation Device (FED): A flexible open-source device for measuring feeding behavior. *J. Neurosci. Methods* **267**, 108–114 (2016).