

Open-field Behavioral setup

In this protocol, the open-field behavioral setup is used to allow freely-moving mice to perform two-alternative forced choice tasks. Below, we describe the protocol to implement an open-field behavioral setup for a visual discrimination task.

Hardware parts list

Electronics

#	Item	Qty	Part #	Vendor	Ind. Price	Notes
1	Arduino Nano Every Board	1	ABX00028	https://store.arduino.cc/products/arduino-nano-every?srsltid=AfmBOooybjmD9E3Ek9i5ffcLJ4FCZAH71edlutPHJ09_QGAqlFubebp4	14,5€	
2	PCB controller Board	1	custom design	JLCPCB (JiaLiChuang (HongKong) Co., LTD)	1,4€	
3	13.3inch Capacitive Touch Screen LCD	1	13.3 inch HDMI LCD (H)	https://www.aveshare.com/13.3inch-hdmi-lcd-h.htm	144€	Presentation of stimuli, with Toughened Glass Cover, 1920×1080, HDMI, IPS
4	Ugreen Cable HDMI M/M 2m 4K/60Hz ED015	1			11,90€	Connects the screen with the PC

Other Hardware

#	Item	Qty	Part #	Vendor	Ind. Price	Notes
1	V-SLOT 2020 1500mm	1	27-99920208	https://grobtronics.com/v-slot-2020-natural-anodized.html?variation_id=13429	14,80€	cut into 4 pieces of 31 cm length
2	V-SLOT 2020 250mm	4	27-99920210	https://grobtronics.com/v-slot-2020-natural-anodized.html?variation_id=13426	2,5€	
3	V-SLOT 2020 500mm	2	27-99920209	https://grobtronics.com/v-slot-2020-natural-anodized.html?variation_id=13427	4,9€	
4	Inside Hidden Corner Bracket 3-Way	4	14-00015421	https://grobtronics.com/inside-hidden-corner-bracket-3-way.html	1,6€	
5	RatRig Cast - 90 Degree Corner Bracket	4	27-22280012	https://grobtronics.com/cast-90-degree-corner-bracket.html	0,6€	
6	Plexiglass plate	7		local vendor	2€	2 33x33cm plates 1 30x30cm plate 4 35x30cm plates
7	Rat Rig V-Hive Enclosure Base Model	1	HW3303GK	https://ratrig.dozuki.com/Guide/01.+	118€	Insulation of light and sound

				V-Hive+Enclosure+Base+Model/183?lang=en		
8	ISOLFON foam plate			https://www.muziker.com/mega-acoustic-pa-mp-5-50x50x5-dark-grey		Sound insulator
9	M4 Screws		M4X10		~0,30€	11x L10 16x L8
10	M4 T-nuts 20x20	9	14-00085144		0,20€	
11	V-Slot Gantry Set 2020 with Three Wheels	2	14-00020155	https://grobtronics.com/v-slot-gantry-set-2020-with-three-wheels.html	9,90€	
12	Lickport	1		Custom		Delivers water
13	LHD Series 3-Way Control Solenoid Valve	1	LHDA0533415H	LEE SLR https://www.theleeco.com/product/lhd-series-3-way-control-solenoid-valve/	93€	

3D printed parts

#	Item	Qty	Filename	Notes
1	Lower corners	4	open-field_bottom_corner.stl	
2	Upper corners	4	open-field_top_corner.stl	

Step-by-step assembly instructions

The open-field behavioral setup is enclosed in the Rat-Rig V-Hive Enclosure Base Model ([Other Hardware list](#), item #7). Assembly instructions can be found [here](#).

Sound-proof insulation

Step 1: To make the enclosure sound-proof, use sound insulation material (eg. ISOLFON foam plate, [Other Hardware list](#), item #8) to cover all sides of the enclosure, i.e. use 4 pieces with dimensions 50x50cm and 2 pieces with dimensions 50x43cm.

Arena

The interior of the enclosure consists of a plexiglass box (arena), where the mouse is placed during the experiment (base: 30x30cm, walls: 35x30 cm, [Other Hardware list](#), item #6).

Step 2: Glue (e.g. hot glue) each side of the arena and place 3D printed parts ([3D printed parts list](#), items #1,2) on the upper and lower corners (Fig. 1A).

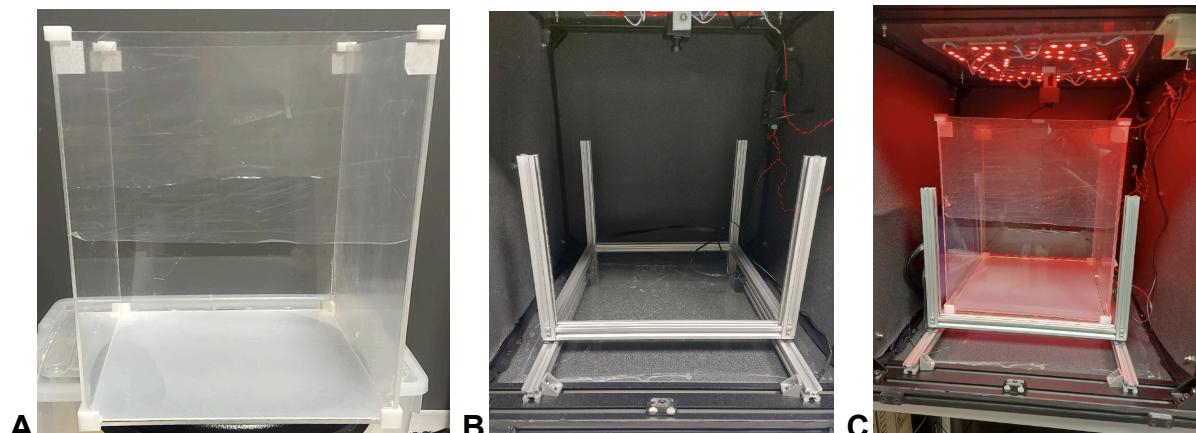


Figure 1. Open-field arena. A. The arena, B. the base of the arena, C. the arena on top of its base and inside the enclosure.

Step 3: Construct the following drawer to place the arena (Fig. 1B, 1C):

- Base: 4 V-SLOT 2020 ([Other Hardware list](#), item #1) with a length of 31 cm each. Connect the rails in the shape of a square and place a plexiglass (33x33cm, [Other Hardware list](#), item #6) in the middle. When connecting the plexiglass to the square, you need to cut its corners at around 6.1 mm.
- Columns: 4 V-SLOT 2020 250 mm ([Other Hardware list](#), item #2). Connect them on the base.
- For the connections of the base and columns, use Inside Hidden Corner Brackets and M4 (8mm) screws ([Other Hardware list](#), items #4,9).
- Rails: 2 V-SLOT 2020 with a length of 50 cm each ([Other Hardware list](#), item #3). Place the rails on the base of the box and screw them using 4 90-Degree

Corner Brackets ([Other Hardware list](#), item #5). To screw the brackets on the V-SLOTS, use M4 screws (10mm, [Other Hardware list](#), item #9) and M4 20x20 Tee Nuts ([Other Hardware list](#), item #10).

- **Sliding:** Screw 2 V-Slot Gantry Sets 2020 with Three Wheels ([Other Hardware list](#), item #11) on each side of the base with M4 (8mm, [Other Hardware list](#), item #9) screws and M4 20x20 Tee Nuts ([Other Hardware list](#), item #10). Attach each V-Slot Gantry Set to the rails.

Screen

Step 4: Place a screen ([Electronics list](#), item #3) on the one side of the box between the 2 V-slots (see Fig. 2A).

Step 5: Connect the cable for power (included in the 13.3inch Capacitive Touch Screen LCD, [Electronics list](#), item #3) and the HDMI ([Electronics list](#), item #4) (Fig. 1B).

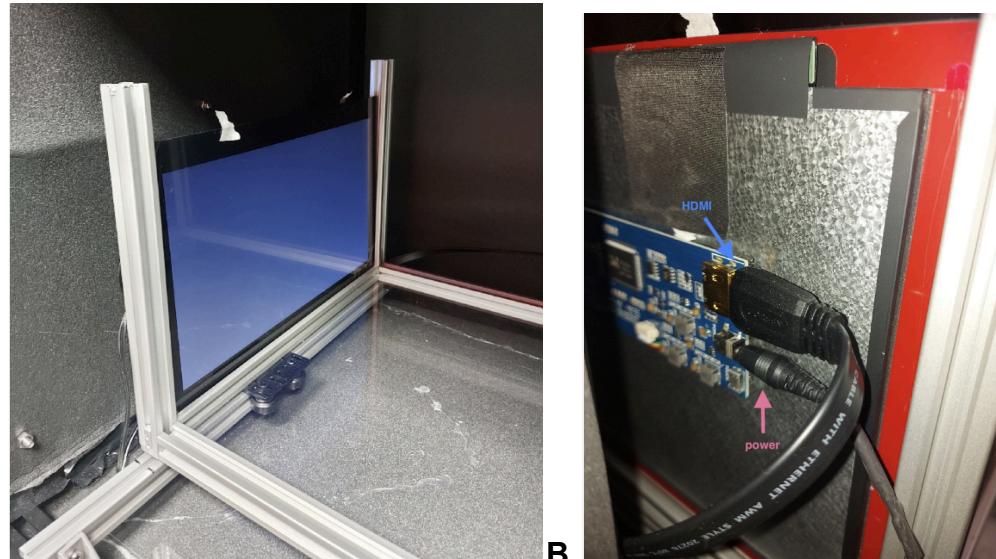


Figure 2. Screen. A. front side of the screen,B. back side of the screen and connections.

Arduino Board

Step 6: Follow instructions for [EthoPy_Controller](#).

Lickport

Step 7: Follow instructions [here](#) to make the lickport ([Other Hardware list](#), item #12).

Step 8: Connect the lickport to the arduino board and a solenoid valve ([Other Hardware list](#), item #13) in a pressurized tubing network (STAR methods, liquid delivery).

Other

To record the experiment you need red LED lights and a camera (we used Arducam 2.3MP AR0234 Color Global Shutter USB 3.0 Camera Module-With Enclosure). If you use a camera that records in darkness, lights might not be necessary.

PC

For the *Open-field* setup in the *EthoPy* project, in addition to the standard [installation](#), you will need to enable real-time pose estimation by installing [DeepLabCut-Live](#), which provides a lightweight, low-latency inference pipeline for online tracking. To achieve frame rates greater than 30 FPS, we strongly recommend using a system running Ubuntu paired with a powerful NVIDIA GPU (minimum 8GB VRAM), as this ensures compatibility with TensorFlow and allows for GPU-accelerated inference. CPU-only systems or unsupported GPUs (e.g., Intel or AMD) will not meet the real-time demands.