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ANY-maze Protocol (Elevated Plus Maze) v6.2

Forked from ANY-maze Protocol (Elevated Plus Maze) v6.2

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ABSTRACT

This protocol is for running rats in the Elevated Plus Maze with ANY-maze software analysis of the experimental data.

TECH SUPPORT OPTIONS

Under the tab titled *Support* in ANYmaze project files can be sent to ask software support questions

Email contact from training:
Emmanuel G. Vlamakis (Software Engineer)
Email: Emmanuel@StoeltingCo.com

Public link for tech support
http://www.anymaze.co.uk/support.htm

PROTOCOL CITATION

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FORK NOTE

FORK FROM

Forked from ANY-maze Protocol (Elevated Plus Maze) v6.2, Cristina Corral

KEYWORDS

any-maze, elevated plus maze, EPM

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39505

BEFORE STARTING

Measure the length of the maze arms and record them (OPTIONAL also obtain an average width of rats measurement)

Record or trim videos so that only 1 rat is in 1 video. The software can look at one continuous video source, but it becomes more difficult to run and more prone to an error of mismatched rat data. There is a video editor on the ANYmaze laptop that can trim and export the clips to the desktop for use.

1 **Open a "New Experiment" from the file tab** and name the protocol and modify settings as described below

(Or open EPM CC, which has all the settings as recommended below)

From the dropdown menu below the protocol title select *Video tracking mode* to record video tracking

From the dropdown menu below the *Video tracking mode* select *Elevated Plus Maze* as the optional image

(If manuel scoring with key inputs is needed instead of ANY-maze tracking, under the take note dropdown in the same dropdown selecting the *TakeNote video observation mode* option is analogous to *Video tracking mode* without ANY-maze tracking)

2 **To add a Video Source** click the plus button titled *Add item* (located in the light gray ribbon bar above the protocol page) and then click *New video source*

- Or right click the tab on the left titled *Video Sources*
- For each video added there should only be for one rat in the video and it should be primarily only footage of the experiment (so around 5-6 minutes long total)

Title the video source (corresponding rat information is recommended, i.e. Video Source r3 for a rat with ID 3 and change the title when the video source is changed)

From the dropdown menu select the last option of *video file* to select a file from the computer

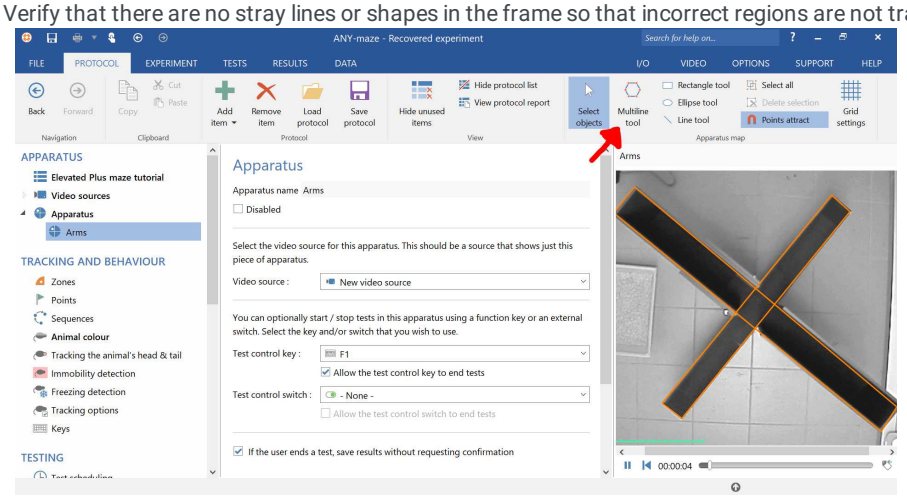
A live camera feed can be used as well if necessary, usually it not needed for EPM
To select the live feed option, from the dropdown menu select the option WEB CAMERA (Port X) with X being the corresponding port for the live camera feed

3 **To add an Apparatus** (a general region of interest) click the plus button *Add item* and then click *New apparatus*

- Or right click the tab on the left titled *Apparatus*

Title the apparatus *Arms* (corresponding rat information is recommended, i.e. Arms r3 for rat 3)

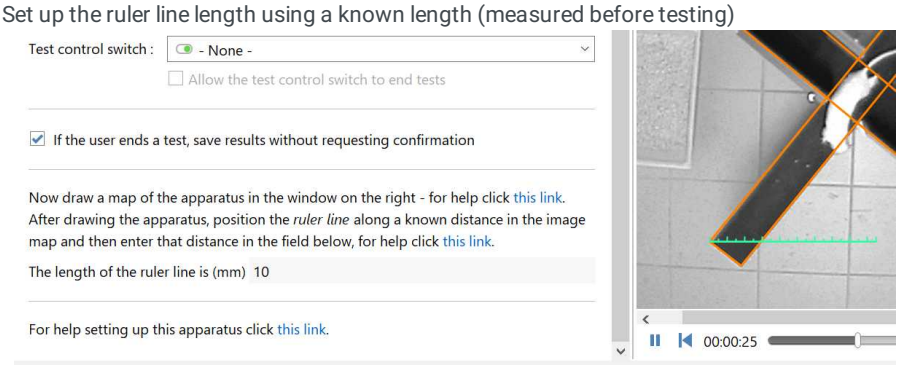
Click a point in the middle of the video and then draw the apparatus map using the multiline tool (double click the mouse to end the multiline shape) to draw the borders for the analysis of the arms
Verify that there are no stray lines or shapes in the frame so that incorrect regions are not tracked



Location of multiline tool and apparatus border

It is recommended that the checkmark at the bottom of the page is checked to save results without requesting confirmation

Set up the ruler line length using a known length (measured before testing)



4 **To add a Zone** (location for analysis,open and closed arms distinction) click the plus button *Add item* and then click *New zone*

- Or right click the tab on the left titled *Zone*

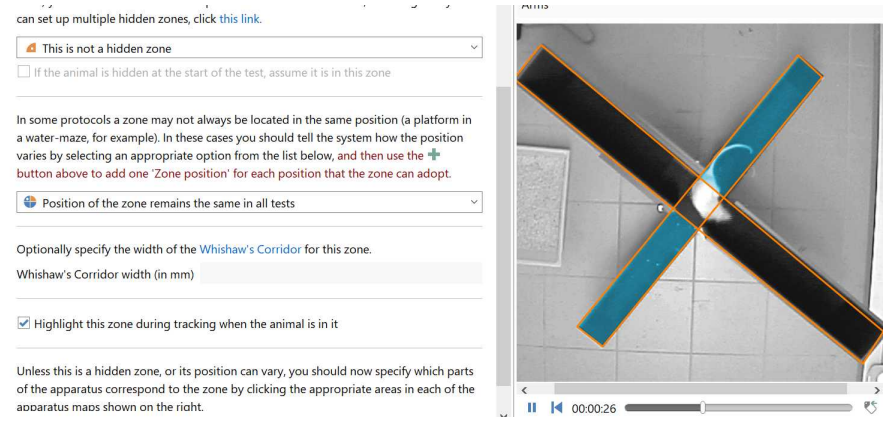
Title the zone *Open arms*

From the dropdown menu select *This is not a hidden zone*

From the dropdown menu below the previous menu select *Position of the zone remains the same in all tests*

Right click the zones of interest to highlight zones when animal is in them

Repeat above steps for the *Closed Arms* zone



5 **To set Zone entry settings**, click on any generated zone (i.e. *Open Arms*) and select *Zone entry settings*

Zone entry follows the *4* paw rule so that 85% of the rat needs to be in a zone for it to be counted under the automatic *Use the center of the animal* setting, but these settings can be adjusted from the tab Zone entry settings

- It can be set to track from the position of the animal's head or the animal's entire area with a manual setting of the minimum and maximum percentage of the animal that must be in a zone
- Based on testing using in lab videos the Center of Area tracking setting is the most accurate for tracking in the open arm and closed arm regions, but not for tracking events such as entering and exiting the arms

6 **OPTIONAL: To add Points** (i.e. the ends of the plus maze) click the plus button *Add item* and then click *New Point*

- Or right click the tab on the left titled *Points*

- It is a measure of the time spent in proximity to a point, so it can be used to note when the rats reach an end of the maze (1 point created for every end, so a total of 4 points)

7 **Select the animal color** by right clicking the tab on the left titled *Animal Color* to select if the animal color will be lighter or darker than the apparatus background
- Contrast is how the software analyzes the videos, so a dark rat on a dark colored maze will not work

8 **Select to track the animal's head and tail** by clicking the tab on the left titled *Tracking the animal's head & tail* and select *Yes, I want the animal's head and tail to be tracked*

By selecting this option the file is larger, but the test will not have to be rerun if you would like to change any track parameters

9 **To add Stages** (set the time of the experiment) click the plus button *Add item* and then click *New Stage*
- Or right click the tab on the left titled *Stages*

Set the test duration for *5 min*

Select to *Perform each trial for all the animals before starting the next trial*

10 **Set the automatic starting of tests** by clicking the tab on the left titled *Automatic starting of tests* and select *Yes, automatically start tests*

11 **Set what to record in the test** by clicking the tab on the left titled *What to record while testing* and from the dropdown menu select *Record a maximum of 10 positions/second* and in the 2nd drop down menu about video quality select *Medium quality*

12 **Set what to display while testing** by clicking the tab on the left titled *What to display while testing* and select *List any active zone*
- Can be adjusted depending on what information is needed

13 **Set the treatment groups and number of animals** by clicking the tab on the right titled *Treatment groups*

Select the *Use Treatment groups* checkmark

Select the button *The user will manually assign the animals to their groups*

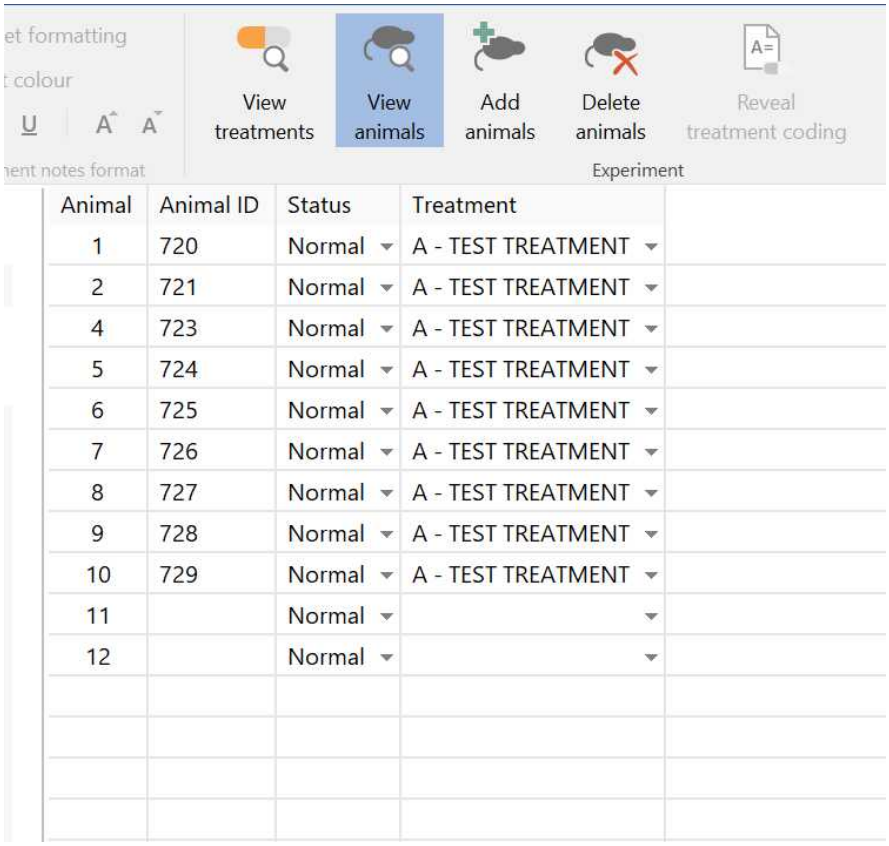
RECOMMENDED: To use established Animal ID codes to identify the animal, click the tab on the right titled Animal ID and click the checkmark *Use my IDs to refer to Animals*

Next click the tab on the top gray bar titled *Experiments* (to the right of the *Protocol* tab)

Click the orange pill icon titled *View treatments* on the top

Add each treatment under the *Name* column and under the *No. animals* column add the number of animals being tested with the corresponding treatment

Click the rat icon titled *View animals* (next to *View Treatments*) on the top to assign the status of the rats and the treatment group from the drop down menus
- To add or delete animals use the corresponding icons on the top bar next to the *View animals* icon, however a deleted animal will still be counted in the total Animal number, so it is recommended to edit an existing animal instead of deleting a rat
- For example, Rat Animal number "3" was deleted (Animal ID number 722), but adding new animals (n=2 added) adds them to the end of the available animals, so unassigned rats animal numbers "11" and "12" were added with rat "3" still being deleted, so the total amount of rats is 11, but the animal number max is 12



Animal	Animal ID	Status	Treatment
1	720	Normal	A - TEST TREATMENT
2	721	Normal	A - TEST TREATMENT
4	723	Normal	A - TEST TREATMENT
5	724	Normal	A - TEST TREATMENT
6	725	Normal	A - TEST TREATMENT
7	726	Normal	A - TEST TREATMENT
8	727	Normal	A - TEST TREATMENT
9	728	Normal	A - TEST TREATMENT
10	729	Normal	A - TEST TREATMENT
11		Normal	
12		Normal	

EXAMPLE: Rat Animal number "3" was deleted

14 **To run multiple tests at once** set up a new video source and new corresponding apparatus (as the plus may be shifted) and in the zone section select the zones to be tracked for the new apparatus (but do not add new open and closed arm zones)

A maximum of 4 tests at once is recommended as more tests cannot be easily viewed on one laptop screen at once

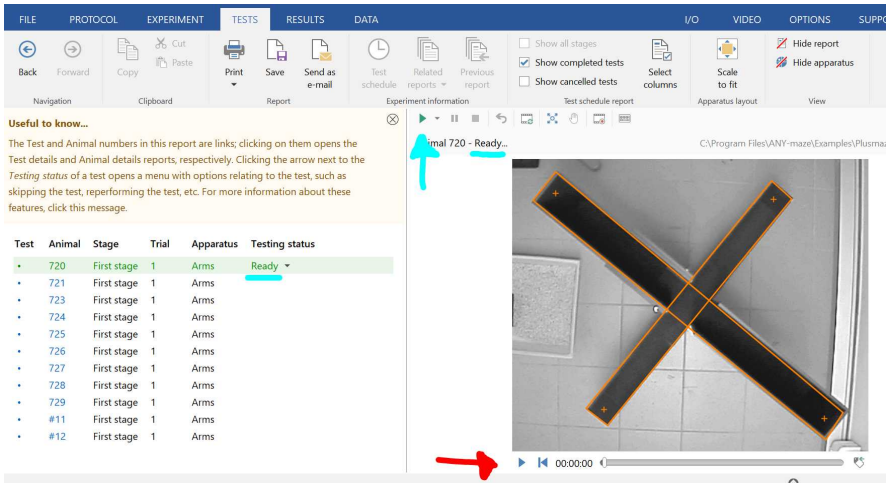
Change the title of the video source and apparatus of each rat when the video source is changed to avoid confusion

15 **OPTIONAL: To make changes to software terminology** click the tab on the top titled Options and on the left side click Terminology

16 **Start the experiment** by clicking the tab on the top titled *Tests*
(If running multiple video sources at once see notes at the end of this card first)

For any test ensure that the apparatus is in the correct location (select a time in the middle to see if it was moved in the beginning before the test was run) and that the zones are properly designated, if they need to be adjusted the test must be stopped and run after the adjustments

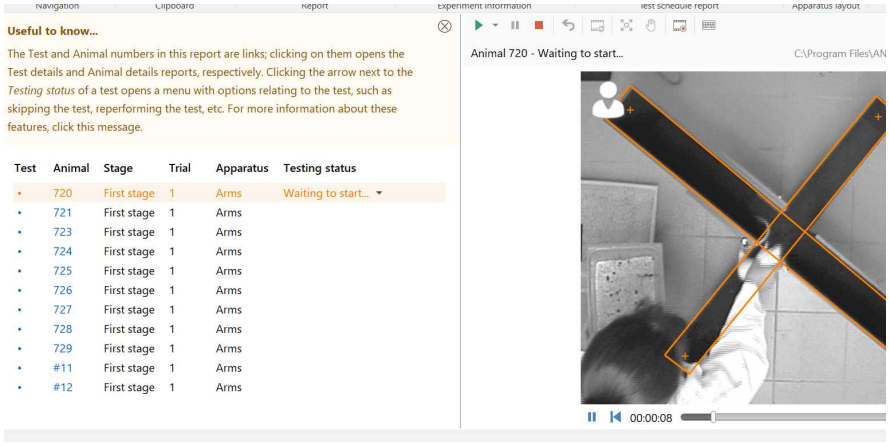
To each video source click the green play button (cyan arrow in image) once to change the testing status from *Ready* (green highlight over text) to *Waiting to Start (*orange highlight over text*)*



Ready

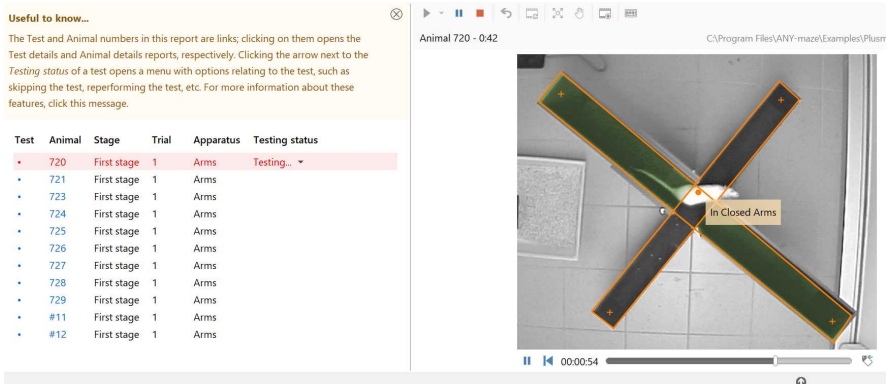
Click the blue play button (Red arrow in image) to begin the video source

The test should not automatically begin if the experimenter is still in frame (a person icon in the upper left hand side should be present until the experimenter has left), if it does then click the red square stop button to redo the test



Experimentor icon in the upper left corner of the video source

Once the experimenter has left the frame the test should begin (indicated by the red highlight over text, the tracking of the animal, and testing status listed as Testing...)
If the test does not automatically begin, scrub the video playback to where the test begins and click the green play button a second time to manually begin the test



Testing phase

If the test needs to be stopped, click the red square stop icon and if the test needs to be rerun click from the Testing

status drop down menu *Rerun test*

Once the test is completed it will say *Completed* under the Testing status dropdown menu and it will be in the *Ready* status for the next rat in the list

Multiple video sources at once:
If there are multiple video sources, select the green plus icon next to the green play button icon to select the corresponding rat (via their animal ID) to the video source and set up each test individually to ensure that they are working for around 30 seconds before setting up and starting the next one (Select *scale to fit* to view the entire video source on the screen)

17 **Results and Data** are available by clicking the tab on the top titled *Results*

Results can generate reports of data, such as data significance and preliminary graphs

- Select the results needed and then on the top gray bar select the icon *View the report*
- This section has optional data tools such as Text (shows data collated by groups to show amount of subjects, SD, mean, etc), Graph (shows line, column, or scatter graphs), or statistical (shows statistical analysis of results)

The tab on the top titled *Data* can generate spreadsheets to copy and transfer data to other data processing software,

To generate a spreadsheet from the Data tab click *Select data* (if greyed out then already on the page) on the top gray bar and select the data then select the *View spreadsheet* icon next to the Select data icon to verify that the data that was selected in the results tab is available to save as a spreadsheet

- If data is missing or not needed go back to the results tab on the top to edit the results to include by selecting the checkmark boxes

To save the spreadsheet select the *Save* icon on the top gray banner and the file can be saved as CSV file to be opened in excel or PRISM for further statistical analysis

18 **For miscellaneous potential issues** see the *Help* tab in the rightmost top corner for an ANY-maze reference guide and the Support tab to contact technical support, see what drivers are installed, and to check for software updates (also see this protocol description)

The *Options* tab is used for sound, appearance terminology, User management, administrative options, and printing options