

Instructions for running an elevated plus maze experiment.

1. The week before bring your mice to the Light Hall vivarium or to the MNL housing rooms.
2. Familiarize yourself with the equipment and software (steps 6 through xx below with a practice copy of the software).
3. Turn the computer sound off.
4. Practice handling the mice and placing them on the maze. This may take several dozen or several hundred mice, but it is essential to the success of your experiment that all mice are placed on the maze in an identical, **non-stressful** manner.
5. The day before behavioral testing, **mark the tails of your mice with an indelible marker**, e.g., zero through four stripes per mouse. Make sure the mark is intense enough to last to the next day. The reason for the mark is so that you can identify the mouse quickly and easily without scruffing or excessive handling.
6. Make a **copy** of the folder “**Image EP Template**” and give it a name that conveys the essence of your experiment.
7. Make sure the computer sound is off.
8. The Image program uses black/white contrast to detect the mouse, and the background is white. So before you start remove anything dark from around the maze.

NB: Nearly every parameter in this program can be changed post-hoc and the data can be re-analyzed ad nauseam with different parameters. One exception is the contrast, which can't be changed too easily. Thus it is important to make sure the contrast between mouse and background are good, both here and in Step 21 below.
9. Open your folder and double-click on the “**Image EP 2.13**” file.
10. Under the “Special” menu choose “Start Capturing”. You should see a live video picture titled “Camera (Live)” if everything is working correctly.
11. Set the camera to a level at which you can see the entire plus maze within the frame. In Light Hall 847E this is “**EP**” level if the maze is on the floor, or “**WM**” level if the maze is sitting atop the water maze.
12. The maze arms should be parallel or perpendicular to the video frame.
13. Close the “**Camera (Live)**” window without saving the changes.
14. Place an **artificial mouse** on one of the arms of the maze. An artificial mouse is a black object the size of a mouse.
15. Under the “**Special**” menu choose “**SetField1**”. A small picture of the elevated plus maze will appear in a window titled “**Field**”, with instructions to draw a box around the maze using the **Rectangle Tool**.
16. Draw a box around the maze using the **Rectangle Tool** (click, hold, and drag). If you're not satisfied with the rectangle you drew, you can draw another one, and another, *ad nauseum*.

Troubleshooting:

If you try to draw a box and nothing happens, it's usually due to one of two reasons. Most commonly, there is already a rectangle drawn around the entire frame (**USE THIS DEFAULT!!!**). This is the default when you open the window, so if you click hold and drag to draw a rectangle nothing will happen. If this happens simply click once inside the frame and the default rectangle will disappear. The other reason you can't draw a rectangle might be that the **Rectangle Tool** is not selected. This happens when you go back to SetField1 after adjusting the **Threshold** (step xxx below), and the **Threshold Tool**

remains selected in the **Tools window**. To fix this, simply choose the **Rectangle Tool** in the **Tools window**.

17. When you're satisfied with the rectangle you've drawn, choose "**SetField2**" under the "**Special**" menu.
18. Now make another rectangle, only covering the center platform of the maze (i.e., excluding the arms), as in Figure xxx. You may repeat this as necessary until you're satisfied with your rectangle.
19. When you're satisfied with your rectangle, choose "**SetPlatformPosition2**" under the "**Special**" menu.
20. Under the "Options" menu choose "Threshold". You'll notice that the video image turns into a silhouette and the Threshold Tool is automatically selected. You'll also notice that the LUT window changed from shades of gray to black and white. The point at which the black turns to which can be adjusted by clicking on that point and dragging. Try it and see what happens to the silhouette in the "**Field**" window, and watch the Threshold level change in the "Info" window. Move it up and down! Don't be afraid!
21. When you're comfortable with how the **Threshold Tool** works, adjust it to a level at which the artificial mouse is a single entity, or "**particle**", and everything (or nearly everything) around it has turned white. (The plus maze itself is black, of course, so it's impossible to make it disappear completely. That's because the maze was built before we started using the NIH Image macro. Someday an enterprising young graduate student will take the initiative to paint it white.)
22. When you've moved the threshold to a level at which the artificial mouse is a single particle and everything else around it has disappeared, make a note of the **Threshold value** in the "**Info**" window. You will need this value later on.
23. Under the "**File**" menu choose "**Quit**", and do not save changes to the "Field" window.
24. Take the **artificial mouse** off of the maze.
25. Double-click on the "**Image EPC 2.03sr**" file.
26. To start collecting data, choose "**CaptureTrials**" under the "**Special**" menu.
27. In the dialog box, give your session a name that is meaningful to your experiment.
28. Over the next few dialog boxes you will have the opportunity to set the parameters for your experiment. Most of them will be pre-set in the "**Initial Parameters**" file in the "**Parameters**" folder, but it's a good idea to check them all first to make sure they're what you want before you start collecting data.
29. At the **first parameters dialog box** you will set the "**Rate**", "**Duration**", "**Bin**", and "**Level**". The **rate** is frames per second and is typically 1.0. To set or check this, type "1" in the dialog box and press "Enter". The default **rate** will be shown and you can accept it or change it. When satisfied with the **rate** press "Enter" and the first parameters dialog box will re-appear.
30. The **duration** is the length of the session in seconds and is typically 300. To set or check this, type "2" in the dialog box and press "Enter". The default **duration** will be shown and you can accept it or change it. When satisfied with the **duration** press "Enter" and the parameters dialog box will re-appear.
31. The **bin** is the block of time you want your data stored in, in seconds, and is typically 60. To set or check this, type "3" in the dialog box and press "Enter". The default **bin** will be shown and you can accept it or change it. When satisfied with the **bin** press "Enter" and the parameters dialog box will re-appear.
32. The **level** is the Threshold value you wrote down in Step 22, and will have to be entered anew for each session. To set this, type "4" in the dialog box and press "Enter". The default **threshold** will be shown and you can accept it or change it. When satisfied with the **threshold** press "Enter" and the parameters dialog box will re-appear.

33. When you are satisfied with all the parameters in the **first parameters dialog box**, type “0” in the dialog box and press “Enter” to be taken to the second parameters dialog box.
34. At the **second parameters dialog box** you will set the “**Subj.**”, “**Frame**”, “**Trace**”, and “**Invert**”. **Subj.** is the size of the mouse on the screen in **pixels**, and typically ranges from 3 to 9999. To set or check this, type “1” in the dialog box and press “Enter”. The default **minimal size** will be shown and you can accept it or change it. When satisfied with the **minimal size** press “Enter” and you will have a chance to enter the **maximal size**. When satisfied with the **maximal size** press “Enter” and the second parameters dialog box will re-appear.
35. The **frame** is the height and width of the plus maze in cm, and is 65 each direction for our acrylic/wood hybrid model. To set or check this, type “2” in the dialog box and press “Enter”. The default **frame width** will be shown and you can accept it or change it. When satisfied with the **frame width** press “Enter” and you will have a chance to enter the **frame height**. When satisfied with the **frame height** press “Enter” and the second parameters dialog box will re-appear.
36. The **trace** option is whether you want the program to record a trace of the session. This is typically set to “0”, meaning off, but it’s experimenter’s preference. To set or check this, type “3” in the dialog box and press “Enter”. The default **trace** status will be shown and you can accept it or change it. When satisfied with the **trace** status press “Enter” and the parameters dialog box will re-appear.
37. The **invert** option determines whether you want black on a white background or white on a black background. This is typically set to “0”, meaning black on a white background. To set or check this, type “4” in the dialog box and press “Enter”. The default **invert** status will be shown and you can accept it or change it to “1” for white on a black background. When satisfied with the **invert** status press “Enter” and the parameters dialog box will re-appear.
38. When you are satisfied with all the parameters in the **second parameters dialog box**, type “0” in the dialog box and press “Enter” to be taken to the third parameters dialog box. The only parameter to be changed in the third parameters dialog box is “Human Counting”. The **human counting** option determines whether you a human will also be tracking the behavior of the mouse in real time. This is typically set to “0”, meaning no human counting. To set or check this, type “1” in the dialog box and press “Enter”. The default **human counting** status will be shown and you can accept it or change it to “1” to turn it on. When satisfied with the **human counting** status press “Enter” and the parameters dialog box will re-appear.

To solve the big vs. small image window problem:

In Image EP 2.13, choose “Start Capturing”