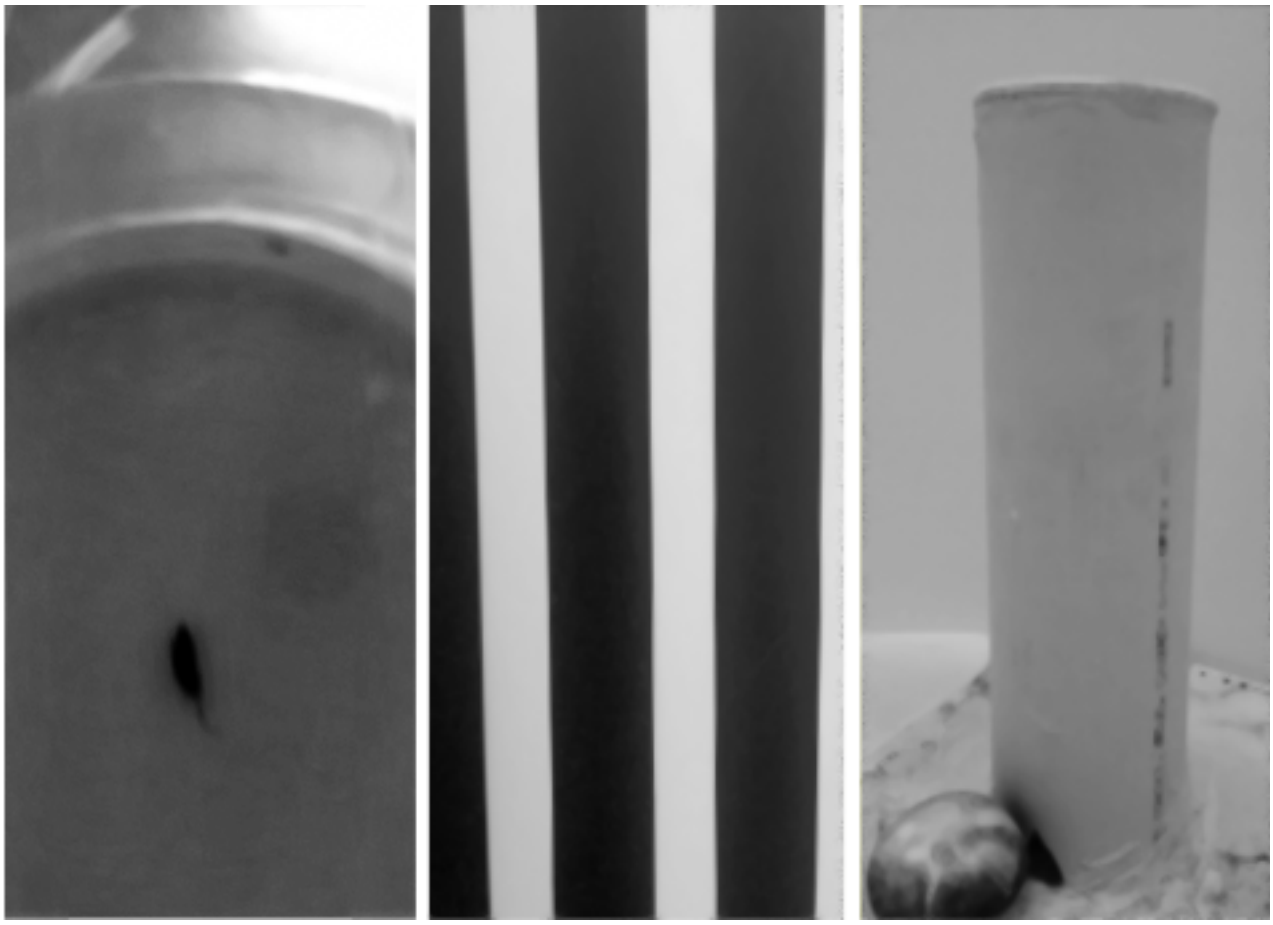
S. BAMJI LAB

Our Guide to the Morris Water Maze



- Shernaz Bamji

The Morris water navigation task, also known as the Morris Water Maze, is a behavioural procedure widely used in behavioural neuroscience to study spatial learning and memory. It can be a very accurate study of learning, memory, and spatial working and can also assess damage to cortical regions of the brain. It is used largely by neuroscientists to measure the effect of neurocognitive disorders on spatial learning and possible neural treatments, to test the effect of lesions to the brain in areas focused on memory, and to study how age influences cognitive function and spatial learning. The task is also used as a tool to study drug-abuse, neural systems, neurotransmitters, and brain development.

Wikipedia, 2015
Click here to visit the source

Things you're going to need before you start:

Platform Comments: Must be decently sized and allow a mouse to sit atop it yet produce enough water depth. Best if it has a flared base.	**Tempera non-toxic white paint** Comments: Mixed with the water to make it opaque and thus the platform invisible in the water.
Flag Comments: Made of a pen taped to flimsy paper with high contrast black and white bars.	**Thermometer** Comments: To measure and thus maintain the temperature of the water between trials.
2+ king size bedsheets Comments: These will form the false walls and enable you to get in and out of the enclosed test space.	**10-quart bucket** Comments: To extract and refill water to the right temperature.
4 spatial cues Comments: Must different overtly. More on them later.	**Water pump** Comments: To remove water at the end of the day.
2+ rolls of duct tape Comments: Comes in very handy, but doesn't adhere to fabric well. Best if it contrasts least with the walls, typically white/off white.	**Garden hose with screw attachment** Comments: To attach to the pump and empty into the appropriate place. May also be needed to fill the pool and screw onto a water source.
Safety pins Comments: Come in handy!	**Laptop** Comments: To record/save videos.
Black Sharpie Comments: To mark their tails and comes in handy.	**Webcam** Comments: To position above the pool and make the recordings.
Paddling pool Comments: To catch some of the overflow from the pool.	**Video recording software** Comments: Must be reliable and fast with enough detail yet small file sizes.
Large mouse pool Comments: Must be marked with N, E, S and W along the edge.	**3+ bath towels** Comments: Again, come in very handy. Water is involved and things happen.
Small fish net Comments: To scoop up animal debris!	**3+ fluorescent light bars** Comments: To provide some light to see the spatial cues without being overbearing.
2 heavy rocks Comments: To hold down flared base of the platform and prevent it from moving.	**Extension lead with 4 outlets** Comments: To extend the reach of the fluorescent light bars.
3+ full 1L bottles Comments: Useful to hold up the false wall if unable to fix it any other way.	**USB extension lead** Comments: To extend the reach of the webcam to your laptop.

Things you shall need from your facility:

Heat mats Comments: To prevent your mice from freezing when they leave the pool. Only one for every 4 recovery cages.	**Recovery cages** Comments: One for every home cage.
Water hose Comments: Attached to a water source that you can use to fill the pool and a drain for when you're pumping the pool.	**Cage changing unit** Comments: Acts as one of your false walls and as your recovery station.
Shelves or a metal frame Comments: Acts as one of your false walls. Good to use as there are many metal bars to fix the fabric.	**Table** Comments: Acts as your recording station. Should be near the entrance to your test room so mice can be seen on your screen as soon as you exit.
Adjustable room lights Comments: You need to be able to turn off the room lights and create your own test room lighting.	

Introduction

What you are aiming to produce is something that looks a little like the setup displayed in Figure 1. The pool is in the centre of a room with large, differing spatial cues on each of the four nearby walls. The pool can be split into four quadrants that may play home to the platform, while the spatial cues mark north, east, south and west of your pool, which is marked also along the lip of the pool. Some space between the pool and the wall allows you as the researcher access to the entire pool without preventing the mice from seeing the spatial cues while inside the pool. This guide is split into three main parts. The first deals with how to set up the Morris Water Maze, the second provides you with a recommended sequence of events/protocol for the test days and the final section examines what to do

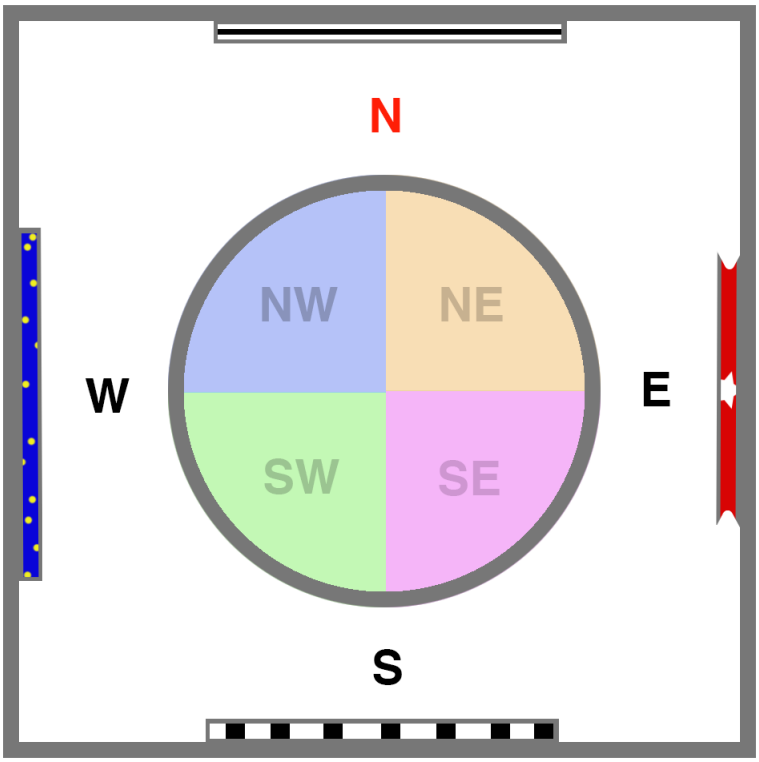


Figure 1. A basic schematic of the ideal Morris Water Maze Setup

with the data produced. I have made attempts at referencing example data sheets (which are either provided within the text or in the appendix; blank data sheets are available in the Tutorials folder on the lab computers) and at creating informative schematics. However, this booklet has been created under the assumption that—at least with the experimental setup—somebody experienced at running the Morris Water Maze will be assisting you. If you have any useful suggestions or edits that you believe would facilitate understanding, be sure to make them known to somebody in the lab!

Setting up the Morris Water Maze

Before entering the procedural space, you shall need to produce four large spatial cues, a platform and a platform flag. Create the flag shown in **Figure 2** from a pen and flimsy card with high contrast black and white bars. It is important that it can be seen from all four sides and that the card is not sturdy enough to support the weight of the mouse, as they will attempt to climb it. The spatial cues should be large and obtrusive such that they can be seen by the mice while swimming. The recommended size for each spatial cue is around 8 × 12 inches. The platform needs to have a large base to steady it in the pool and to be just large enough for a mouse to sit on. Consider the depth of your pool when judging the height of your platform and leave a couple of inches to prevent the mice from leaping from the pool. To help the mice sit comfortably on the platform, white felt can be used to coat the top.

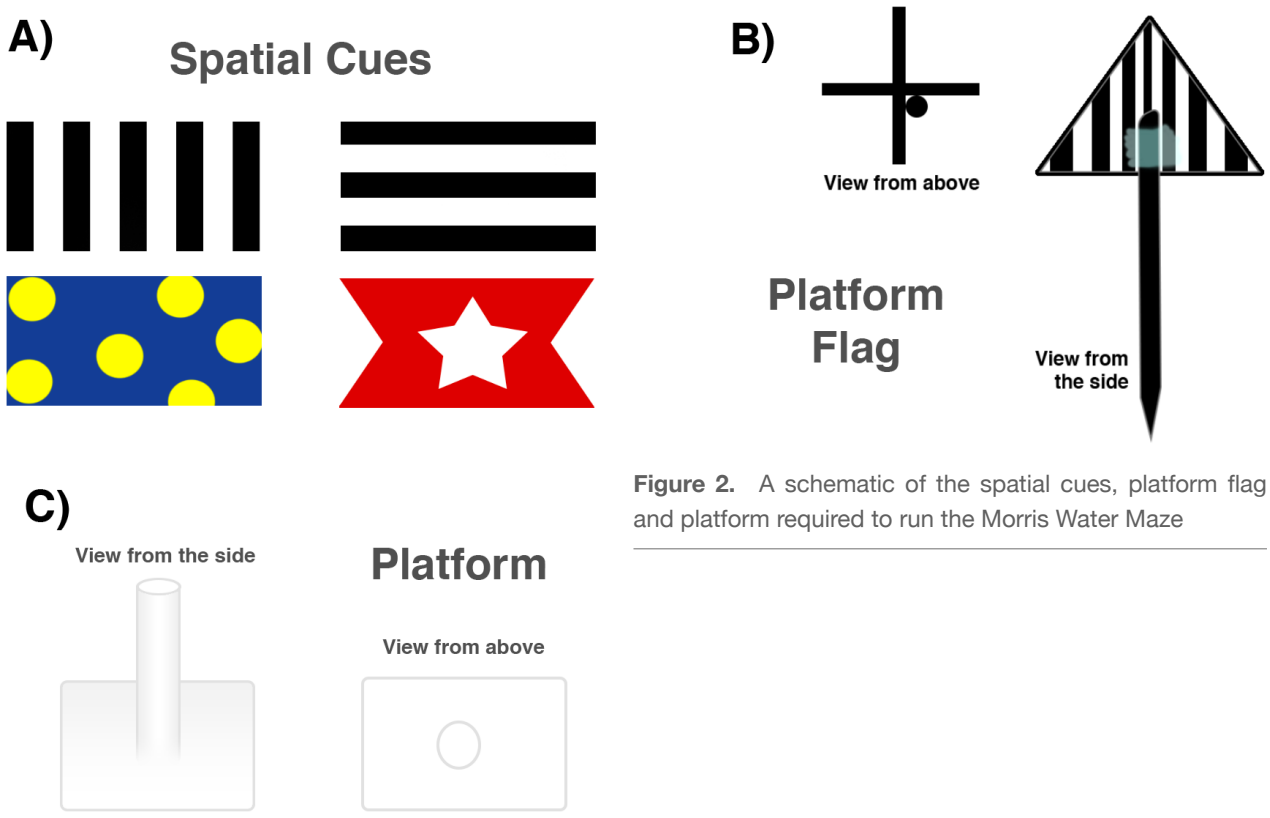


Figure 2. A schematic of the spatial cues, platform flag and platform required to run the Morris Water Maze

Start by clearing a large space in the procedural room and blowing up the paddling pool. It is recommended to use the corner of a test room as this will provide you with two solid walls and thus you need only create two further false walls. Remember that you also need a designated space for the recovery cages and your laptop, preferably as close to the pool as possible. Once the pool is in place, ensure that there is enough room for the researcher to comfortably walk around the perimeter. Next, place a step ladder or foot stool inside the paddling pool and use duct tape to fix a webcam to the ceiling. Be sure to also tape the wire to the ceiling, as fixing the webcam itself can often be tricky and, should it fall, the taped wire should take its weight. Use the USB extension lead if need be to extend the webcam lead to your laptop in its intended place. Load the recording software on your plugged-in computer and ensure that you can see the entirety of the paddling pool before moving on. Put your mouse pool inside the paddling pool and then move your metal frame/cage changing unit into place in order to create your false walls. Cover both of them in a king size bed sheet that is close in colour to the walls. Often, they are white, cream or off white. Fix them into place by typing knots in the fabric to the metal frame and pinning the end to the knot itself. On the cage changing unit, heavy bottles can be placed atop to hold the fabric. Duct tape can be used also, but be warned that it does not adhere well to most fabrics. Where the two fabric walls meet, pin one to the other at a reasonable height to provide a doorway for your to enter and exit the test space. Once your false walls are secure, put up your spatial cues. If you have similar cues (e.g. horizontal versus vertical black and white bars), consider placing them opposite each other. Mice vision is not that acute and having them too close may lead to confusion. Plug in your extension lead and then tape it to the wall. With the water maze, it is always best to assume some water will be spilt. Fixing the outlets low down on the wall tries to prevent water from entering the electrical unit. Remember, if the extension lead wire provides high contrast against the wall, you shall wants to duct tape the wire or tuck it behind one of your false walls. Plug in your fluorescent bar lights and spread them out around the perimeter of the mouse pool. Remember that you don't want to trip over or step on them as you're walking around the maze. Place your platform inside the pool and then secure the base with a heavy rock. As you're filling the pool, the platform may shift. Set up your recovery cages without turning on the heat mats, place three differently coloured towels (in case you dedicate a given purpose to a specific towel, you can easily tell them apart) by your laptop and put your cage trolley in a convenient location. On top of the towels, put your bucket, small fish net, white paint and thermometer. Lastly, check that the water hose in your facility can reach your pool location and that you can turn the lights off. Then you're done with setup! By the end, it should look a little like **Figure 3**.

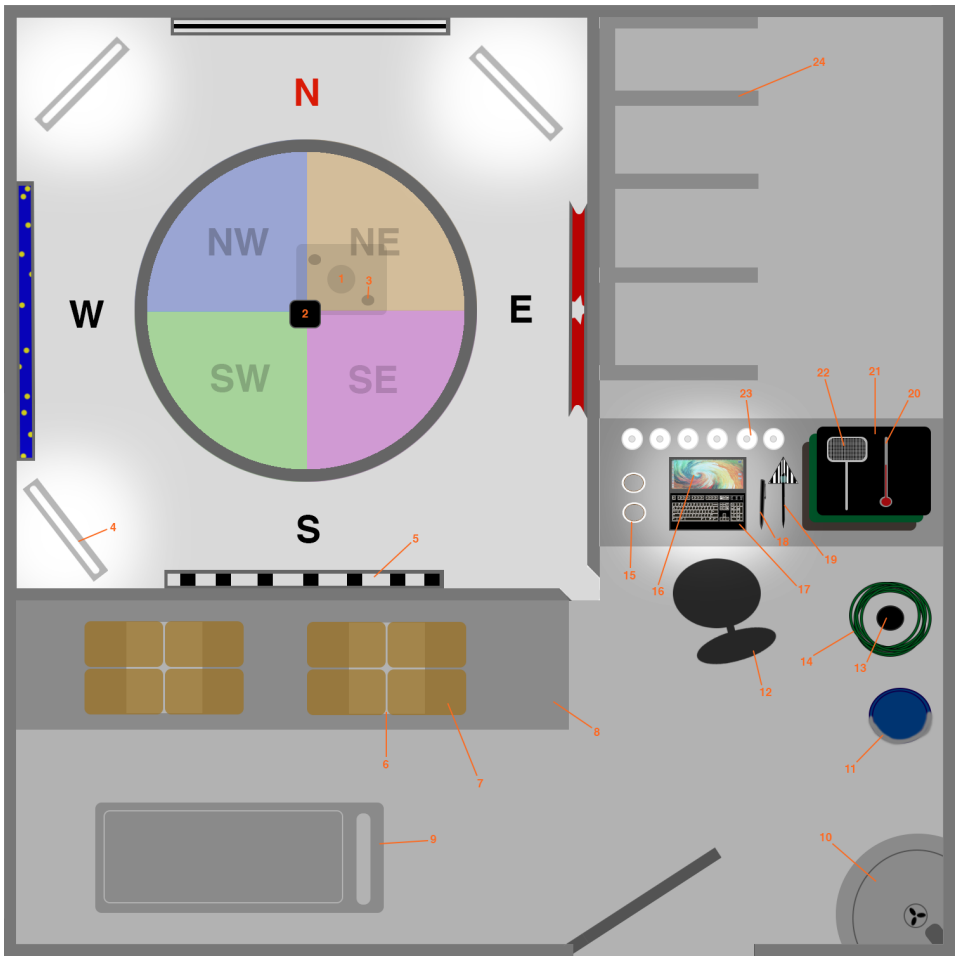


Figure 3. A detailed schematic of the ideal Morris Water Maze setup

1 Platform
2 Webcam
3 Heavy rock
4 Bar light
5 Spatial cue
6 Heat mat
7 Recovery cage
8 Cage changing station
9 Trolley
10 Water source
11 Bucket
12 Chair
13 Water pump
14 Hose
15 Duct tape
16 Recording software
17 Laptop
18 Sharpie
19 Platform flag
20 Thermometer
21 Towels
22 Fish net
23 White paint
24 Metal frame

Running the Morris Water Maze

It is preferable (but not essential) that your test hours coincide with the awake hours of the mice you are testing. For most experiments, this will mean placing your mice on a reverse light cycle. Be mindful of this if you are running an experiment during their sleep phase. There are three main stages to the Morris Water Maze test we run in the Bamji lab. The first phase familiarises the mice with the task and assesses their ability/ motivation to swim and their ability to see the platform flag. Though it is important, it is not part of the Morris Water Maze memory test, so it is considered here as day 0. There are four trials during this initial phase and each trial, the platform is moved to a new quadrant and the mouse starts in pool at a new direction, such that by the end of the day every mouse has entered the pool from N, E, S and W and the platform has been in

the NE, NW, SE and SW quadrants during each of the trials. The second phase, known as the acquisition phase, tests the mice's ability to remember the location of a hidden platform. For four days at four trials per day, the mice are entered into the pool from all four directions (N, E, S and W), but the platform remains in the same quadrant. Each mouse is given a minute to find the platform. If they do not find it before the minute is up, then you must place them on the platform and give them around ~5 seconds to gather their location. If they find the platform before the minute is up, give them ~5 seconds to scan their surroundings and then enter the test room to collect them. On day 5, the mice experience five trials. The first of the day is known as a probe trial wherein the platform is removed entirely and each mouse is given a minute to swim in the pool. In the subsequent four trials—which mark the start of the reversal phase—the platform is moved to a quadrant distinct from the location it was in during the acquisition phase, preferably to the opposite quadrant, such that if it were present in NE during the acquisition phase, the platform would be in the SW during the reversal phase. Four days of four trials in this new location are performed and then the Morris Water Maze is over. A rough timeline is depicted below in **Figure Four**.

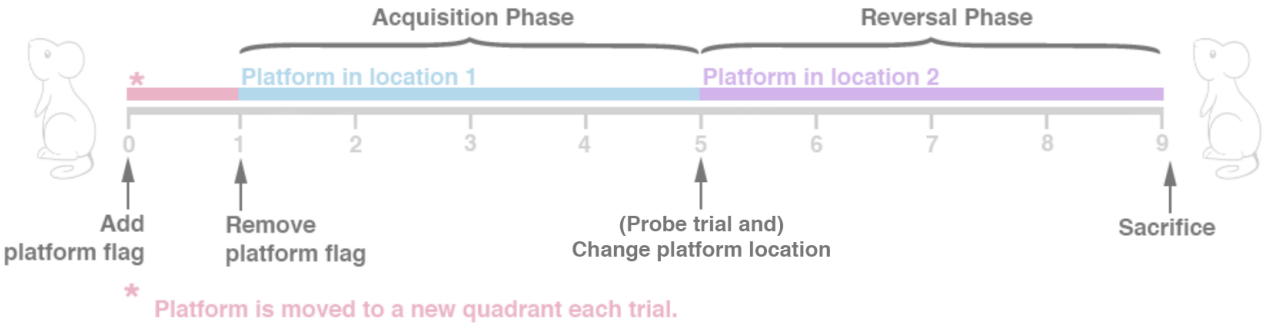


Figure 4. A timeline of the test, acquisition and reversal phases, as well as the probe trial and sacrificable date

Before you start for the day, we recommend first laying out your data table. Examples are available on the lab computer in the Tutorials folder. The table displayed below shows an example data table. The columns represent the trials with the platform location during each trial present in the column header as well as the entry location of the mouse during that trial (shown as a flag). Remember that the mouse must enter at all four locations and it must be in a pseudorandom order that is different day to day.

Animal ID (in order tested)	Trial 1 S Platform in NE	Trial 2 N Platform in NE	Trial 3 W Platform in NE	Trial 4 E Platform in NE
A1				
A2				
B3				
B4				

The coloured groupings of cells (either grey or blue) represent mice that are caged together. The animal ID represents the cage (e.g. **A**) and mouse ID number (e.g. **2**).

Below is a checklist of things you shall want to do as you get in at the beginning of each test day:

1. Make sure that nothing fell down overnight;
2. Set up the webcam on your laptop and check that you can see the entire pool in your video recording software;
3. Glove up and tail mark your mice;
4. Set up the heat pads and recovery cages. Place the recovery cage halfway onto the heat pad;
5. Clear the pool of any animal detritus from the day before;
6. Make sure the pool is orientated correctly with the spatial cues and the platform is in the correct location;
7. Fill the pool with water and ~450ml of white paint. Many animal care facilities will only have hoses attached to cold water and so it may be necessary to add hot water (note: not boiling) using your bucket as it fills;
8. Skim the top of the water with the fish net and remove any debris that floats to the top as it fills;
9. Turn on the fluorescent bar lights on the floor and turn off the main lights;
10. Bring the mice in and give them ~15 minutes to habituate to the room. If you wanted to take a bathroom break or have a drink/something to eat, this may be the best time to do it;
11. Put up a sign on the door instructing passersby that a Morris Water Maze is being performed in your procedural room and that they should not knock or enter unannounced. Instead, provide them with your email address and name should someone need to contact you;
12. Glove up and take the water temperature. You want the pool to be around 26-28°c at the beginning of the trial. Consider that unless you are using a water heater to maintain the temperature it will be cooling as you go and so starting at the higher end of the range is preferable;
13. You are ready to begin taking recordings. Press record, gather your mouse, place it in the water, monitor its progress on your laptop screen, collect the mouse, place it into its recovery cage and stop your recording. Between each mouse, you will want to check the pool for debris which you can remove with your fish net. Repeat until the current trial is complete;
14. Between trials, remove two buckets of water from the pool and then replace it with two buckets of hot water. Take the temperature again and adjust as needed until it is within the 26-28°c range. Once complete, go back to step 13 for the next trial.

At the end of the study, home cage all of your mice going in the same cage order that you use to run each trial (giving any wet mice time to dry off before you home cage

them). Place the mice back in the mouse holding room. Then you are ready to turn on the main room lights and start your cleanup. Make sure the floor is clear of debris/water and turn off the floor lights. Fix your hose to your water pump and place it into the water. Secure the other end of the pump hose to a nearby sink to prevent recoil from causing it to fall out and then turn on your water pump. As the water is being removed (it shall take a while), you can clean the recovery cages, cage changing station and heat mats. Lastly, turn off the pump, fix the hose ends together (some water will still be in the hose pipe and this step prevents it from spilling out) and place things away in their appropriate locations. Then you're all done for the day!

Analysing Morris Water Maze Videos

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