

Your Distributor is:
Braintree Scientific, Inc.
PO Box 850498, Braintree, MA 02185
781-917-9526
Email: Info@braintreesci.com
Web: www.braintreesci.com

Getting started with ANY-maze

Setting up and starting work with ANY-maze



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Stoelting Co.

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Chapter 1

Introduction

What is ANY-maze?

ANY-maze is a flexible video tracking system designed to automate testing in behavioural neuroscience experiments. As such it's ideal for use in studies involving water-mazes, plus mazes, T-maze, light-dark boxes, place preference boxes, etc. etc. - in fact it can be used with almost any type of behavioural testing apparatus and is flexible enough to adapt to new equipment or new testing protocols.

Purpose of this manual

This manual has been designed to help you get started with ANY-maze. It begins by explaining how to set up the physical equipment you will need and then goes on to explain how to use the software itself.

ANY-maze is a complex system and this manual is not intended to be a comprehensive guide to all of its features (for this the system includes on-line help). Rather it's intended to get you to a point where you understand enough about the system to use it with confidence and explore the advanced features at your leisure.

Conventions used in this manual

The following conventions have been adopted in this manual:

- Text in *italic* indicates the names of elements within the software.
- Text in **bold sans serif** indicates the names of fields, buttons, menu options, etc. that appear within the software.
- Text in `fixed width font` indicates something typed into the software by the user.



Context sensitive help can be accessed at any time by clicking the help button.

Getting help with ANY-maze

ANY-maze includes comprehensive on-line help that can be accessed at any time by clicking the **Help** button. This will cause a help topic to be displayed related to the activity you are actually performing. If this message doesn't answer your question you can search the on-line help or you can navigate within the help using the *contents pane*.

Searching the on-line help

1. Select **ANY-maze help and support centre** from the **Help** menu.
2. ANY-maze displays *the Help and support centre* window. Click the **Search the ANY-maze help topics** button, as shown in figure 1.1

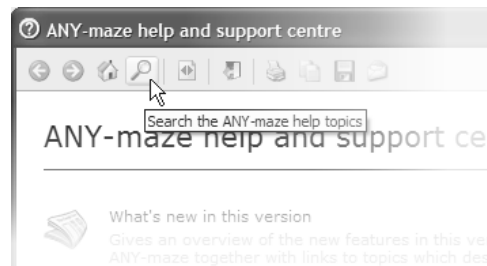


Figure 1.1. The search button can be used to search the ANY-maze help topics.

3. The help topic search page, shown in figure 1.2, will be displayed. Here you can either enter a word to search for, such as “ANOVA”, or you can type in a question such as “How do I print a report”. Either way, after making your entry you should click the **Search Button**.

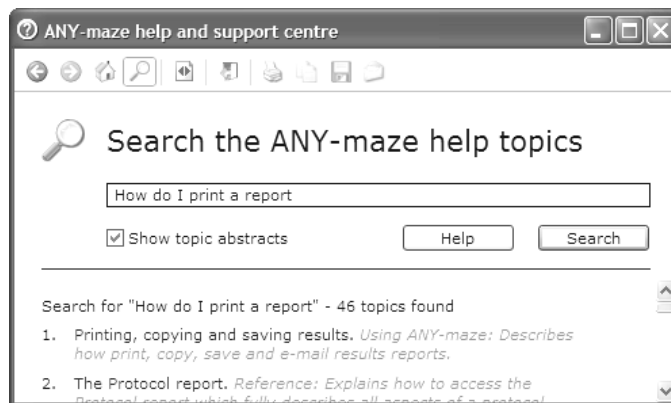


Figure 1.2. The search topics page.

4. ANY-maze will display a list of help topics that may answer your question. Each topic includes a short abstract making it easy to identify the one you want.

Using the contents pane of the on-line help

1. The on-line help *contents pane* is shown on the left side of the *ANY-maze help and support centre* window. This pane may be hidden, but can be shown by clicking the **Show or hide contents** button – see figure 1.3.

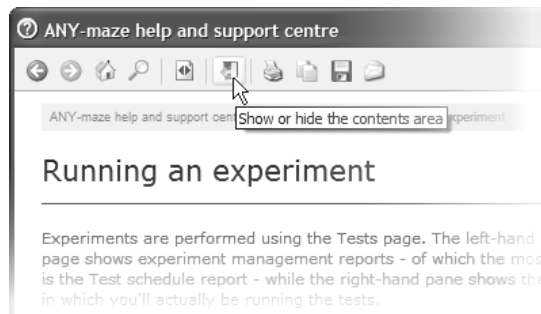


Figure 1.3. The Show contents button can be used to show the on-line help contents pane when it's hidden.

2. The *contents pane* shows the structure of the *help and support centre* topics. You can navigate through the topics by clicking them.
3. If a topic has a + sign next to it then it includes sub topics, clicking it shows the sub-topics list – see figure 1.4.

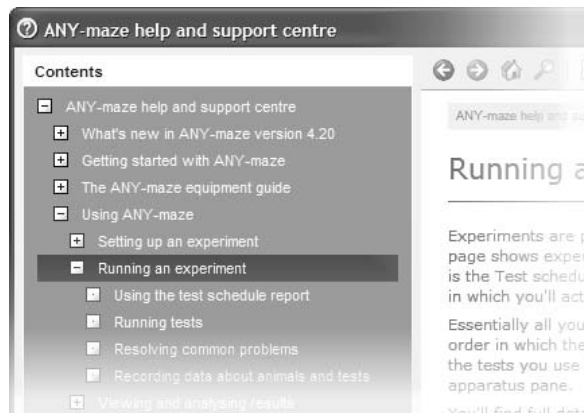


Figure 1.4. Clicking a topic with a + sign shown next to it opens a list of its sub-topics

Contacting ANY-maze technical support

If you can't find the answer to a question in the *ANY-maze help and support centre* then simply send an e-mail with your query to ANY-maze technical support. We're delighted to help with any questions no matter how simple or complex.

The technical support e-mail address is: `techsupport@anymaze.com`

The ANY-maze technical support team is always available to help you with any questions you may have, just send them an e-mail.

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Chapter 2

Setting up ANY-maze

Overview

Before you can start work with ANY-maze you will need to:

- Install the ANY-maze software on your computer.
- Possibly install a digitiser card in your computer.
- Install any driver software required by your camera or digitiser.
- Test that image capture is working correctly.
- License your copy of the software so it will record test results.
- Mount the camera over your apparatus.

You may find it easiest to perform all but the last of these steps *before* actually taking the computer and camera through to your lab - this is particularly true if the computer can't be connected to the Internet in the lab.

Installing the ANY-maze software

Before doing anything else, it's a good idea to install ANY-maze on the computer you intend to use for tracking.

1. Insert the ANY-maze CD into your computer's CD drive. A window will automatically open showing various options; choose **Install ANY-maze on this computer**.
2. The installation is a straightforward process and only takes a few moments. If you want to keep things simple, just select the default option on all the screens.

Running ANY-maze for the first time

After installing ANY-maze you will probably have an ANY-maze icon on your desktop – double click it to start the program.

1. If, during the installation, you specified that ANY-maze should manage its own users (as opposed to using Windows users) then you will be presented with the *ANY-maze log-on window*, see figure 2.1.



Figure 2.1. The ANY-maze log-on window before any users have been set up. Clicking the “I’m a new user button” will setup an initial user.

2. Obviously there will be no users listed, so you should click the **I’m a new user** button.

3. A message will be displayed informing you that you will be an *ANY-maze administrator* – this is because you're the first user of the system. After reading this message, click **OK**.
4. You will now need to specify a user name and password that you will use to access the system in the future. As you will be the *ANY-maze administrator* it's important that you don't forget this name and password – choose something memorable! After entering them click **OK**.
5. A brief welcome message will then be displayed telling you a few key things about ANY-maze, after reading this message press the **Esc** key on the keyboard.
6. Finally, if your computer is connected to the Internet, the system will check the ANY-maze web site for updates to the program that have been released since your ANY-maze CD was produced. If an update is found you should download and install it by following the instructions given.

Preparing your computer to capture images

The next step in setting up your computer is to install your camera or digitiser so that ANY-maze can capture video images. There's no need to do this in the lab.

The following sections describe the basic steps involved in setting up different types of cameras for use with ANY-maze:

USB cameras

If you are using a USB camera you will need to install the driver software provided by the camera's manufacturer. It is usually the case that you need to install the driver before plugging the camera into your computer.

When using a USB camera you normally have to install the driver software before connecting the camera.

FireWire cameras

Installing the FireWire camera driver

You don't need to install any software supplied with your FireWire camera in order to use it with ANY-maze.

ANY-maze is provided with its own driver for FireWire cameras. Therefore it is not necessary to install any software that came with your camera - indeed you should avoid doing this as it may prevent ANY-maze from recognising the camera.

The ANY-maze FireWire camera driver will usually be installed automatically during the overall installation of the program. Nevertheless, it's a good idea to confirm this before connecting your camera to the computer. To do this, select **Preference** from the **Options** menu and then click the **Drivers** tab in the window that appears.

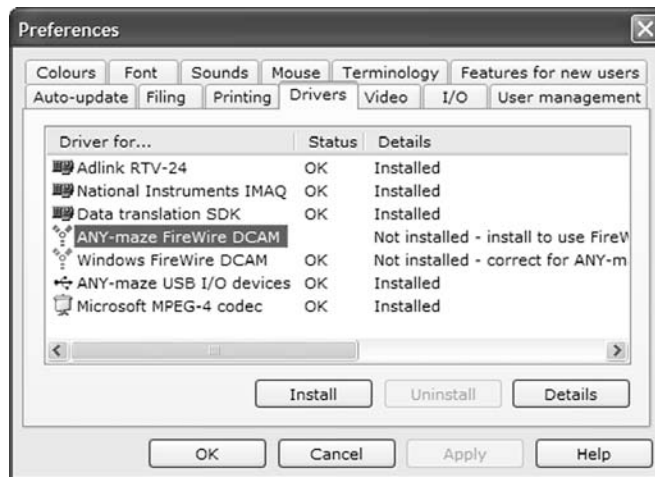


Figure 2.2. The Drivers page of the ANY-maze preferences. In this example the ANY-maze FireWire DCAM driver is not installed, to remedy this you just need to click the Install button.

The Drivers page shows the status of a range of drivers used within ANY-maze including the FireWire DCAM driver. You should confirm that the *Status* for this driver is *OK* and the *Details* read *Installed*. If this is not the

case then click the entry for this driver (to select it) and then click the **Install** button. You should also confirm that no other driver has a *Status* of *Error* – if one does, then the *Details* column will show a recommended course of action.

After checking that the FireWire driver is installed you should connect the camera to your computer.

Powering a FireWire camera

If you are using a FireWire camera with a desktop computer, the computer will almost certainly provide power to the camera through the FireWire cable. However, if you are using a notebook computer the camera will probably need a separate power supply – these are available from Stoelting.

FireWire cameras usually need an external power supply when connected to a notebook computer.

DV camcorders

The driver software for DV camcorders is part of something called Windows DirectX, which is automatically installed by Windows 2000 and XP. Therefore, with these versions of Windows, you don't need to do anything to make a DV camcorder work.

However, if you are using a different version of Windows, you will need to install DirectX from the ANY-maze CD. To do this, simply insert the CD into your CD driver and select **Install Windows DirectX** from the list of options that appears.

Although DV camcorders usually connect to a computer using a FireWire cable, they don't need the FireWire camera driver to be installed.

Analogue cameras

Installing a digitiser

You will probably need to open your computer to install a digitiser. So, you may want to ask your IT support personnel to help with this step.

If you are using an analogue camera with ANY-maze you will need to install a digitiser (sometimes called a frame grabber) in your computer. This is the device that converts the images from the camera into a digital form that ANY-maze can analyse. ANY-maze works with a number of digitisers from different manufacturers.

- ❑ You should install the digitiser by carefully following the instructions provided by the manufacturer.
- ❑ It is often the case that you need to install the driver software for the digitiser before installing the digitiser in your computer.
- ❑ When using a Data Translation digitiser will also need to install the *DT frame grabber SDK*. You can do this from the *Drivers* page of the ANY-maze *Preferences* window (see figure 2.2).

Connecting and powering an analogue camera

Analogue cameras are usually supplied without a cable to connect them to the digitiser. However, Stoelting can provide suitable cables in various lengths.

These cameras also need a separate power connection. Some devices require mains power, while others require 12VDC or possibly 24VAC. Again Stoelting can supply suitable power adaptors if required.

Network cameras

If you are using an Axis network camera with ANY-maze then there is no driver software to install, however, you will need to configure both the camera and ANY-maze before it will work.

- ❑ First set up the camera on your network by following the instructions provided by Axis. Note that ANY-maze uses Universal Plug and Play (UPnP) to detect the camera's IP address so there's no need for you to know this. Therefore, if your network uses a DHCP server, setting up the camera will be as simple as plugging it in.

- ❑ Before using the camera in ANY-maze you should set its Administrator password – this process is described in the Axis instruction manual.
- ❑ Finally you will need to configure the camera in ANY-maze. To do this, select **Preferences** from the **Options** menu and then click the **Video** tab in the window that appears – see figure 2.3.

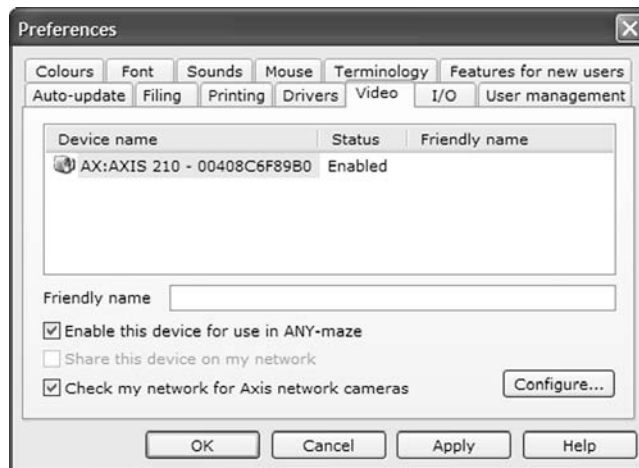


Figure 2.3. The Video page of the ANY-maze Preferences window.

By default, ANY-maze does not detect Axis network cameras so you first need to click the **Check my network for Axis network cameras** button. After a few moments you camera should appear in the list of video devices; click it (to select it) and then click the **Configure** button, this will cause the *Axis network camera configuration window* to open. In this window enter the camera's administrator name and password.

Testing image capture

Having set up your digitiser and/or camera you are ready to test image capture on your computer.

1. Start ANY-maze and, if necessary, log-on.
2. Click the **Monitor** link shown at the top right of the screen – this will switch to the *Monitor page*.
3. On the right of the *monitor page* is a panel titled *Video capture*. Listed in this panel should be at least one entry for your camera or digitiser – see figure 2.4.
4. Click the entry and the video image from your camera should appear.

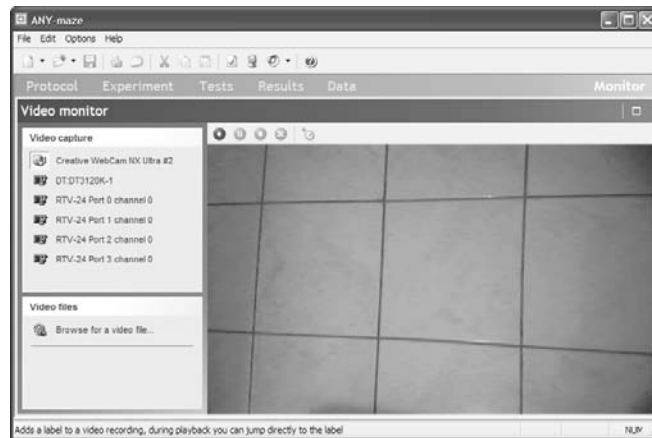


Figure 2.4. The ANY-maze Monitor page. Here the image from the Creative Web Cam NX Ultra #2 is being displayed.

If your camera or digitiser isn't listed in the Video capture panel, or if it is listed but no image appears then:

1. Check all the connections and restart your computer.
2. Search the ANY-maze help for the words "Equipment problems" – this will find a comprehensive troubleshooting topic.

3. Contact ANY-maze technical support, who will be glad to help.

Licensing ANY-maze

After installing ANY-maze on your computer you will find that everything in the program works correctly but, when a test ends, the results are simply thrown away. This is because the copy of ANY-maze is unlicensed.

Until you license your copy of ANY-maze it will discard test results when a test ends.

To license ANY-maze you need to perform the following steps.

1. Start ANY-maze and, if necessary, log-on.
2. From the **Help** menu select **About ANY-maze**.
3. The *ANY-maze system information* page will open.
4. The second section on this page is titled *License information*, and includes an entry for *Serial number*. Note this number and send it to us in an e-mail.

When we receive your serial number, we will create a license number for your copy of ANY-maze and send it back to you by e-mail. You will then need to activate the license as follows:

1. Start ANY-maze and, if necessary, log-on.
2. From the **Help** menu select **ANY-maze help and support centre**.
3. On the *Help and support centre* home page, click the link titled **Purchase, renew or activate an ANY-maze license**.
4. On the next page click the **Activate** link, shown on the left.
5. On the next page type your license number into the boxes and then click the **Activate now** link.
6. A page will be displayed showing that your license has been activated successfully.

License numbers are computer specific, so, if you change your computer, you'll need a new license number.

A few useful things to know about ANY-maze licensing

- ❑ ANY-maze serial numbers are unique to each computer and ANY-maze license numbers are unique to each serial number – for this reason the license number you receive will only work on the computer whose serial number you send us. You should therefore make sure it's the computer you intend to use for tracking.
- ❑ It's easy to transfer a license from one computer to another and there's no cost involved. If you ever need to do this, just contact ANY-maze technical support.
- ❑ You can install unlicensed (free) copies of ANY-maze on as many computers as you like. You won't be able to use these computers to track animals, but you will be able to use them to analyse results, record or view videos, etc.

Setting up ANY-maze in your lab

After installing the software, testing your camera and licensing your copy of ANY-maze, you are ready to actually set up the system in your lab.

Mounting the camera

The first step is to mount your camera so it can see your apparatus. In most cases you will want to mount the camera over the apparatus and it's usually easiest to mount it to the ceiling. Stoelting can provide camera mounts for most situations, including a mount specifically designed for suspended ceilings.

- ❑ Although, it is most common to mount a camera directly above the apparatus looking down, you can also use a camera that's looking at the apparatus from the side. However, in both cases, you should try to ensure that the camera is mounted perpendicular to the apparatus and not at an angle – see figure 2.5. (ANY-maze *will* function with

cameras mounted at an angle but there are disadvantages to doing this and it's not recommended.)

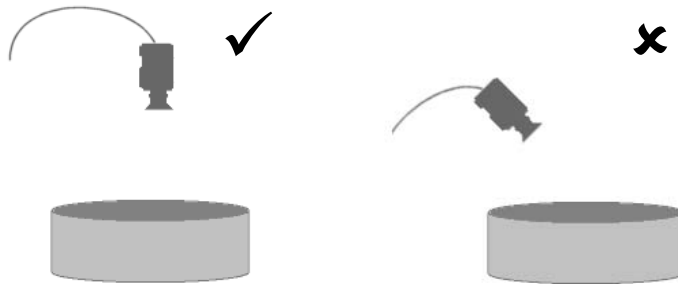


Figure 2.5. If at all possible the camera should be mounted perpendicular to the apparatus.

- ❑ You should try to mount the camera so that it is rigid and won't move or vibrate during tests.
- ❑ It's a good idea to tie back the cable from the camera so it won't be knocked by the experimenter during a test.

You should make sure your camera is mounted rigidly.

Zooming in on your apparatus

If your camera has a vari-focal or zoom lens you should now adjust it so that the apparatus fits the camera's view. It's easiest to do this while viewing the image in the ANY-maze Monitor page (the same page used to test the camera – see above).

- ❑ You should adjust the zoom so that the apparatus is *loosely framed* in the image, i.e. so there's a border all around it – see figure 2.6.
- ❑ If your camera doesn't have a vari-focal or zoom lens then, if possible, you should move the camera and apparatus closer together or further apart to achieve the same goal.

Use optical zoom in preference to digital zoom – the image quality will be better.

- ❑ Even if you can't move the camera or apparatus then, provided the entire apparatus is visible to the camera, ANY-maze will still work.
- ❑ If the apparatus appears small in the image then you can use a digital zoom function within ANY-maze to enlarge it (this process is described in chapter 3). Note, however, that optical zoom (using a zoom lens) is superior to digital zoom so, if you have a zoom lens, use that in preference.

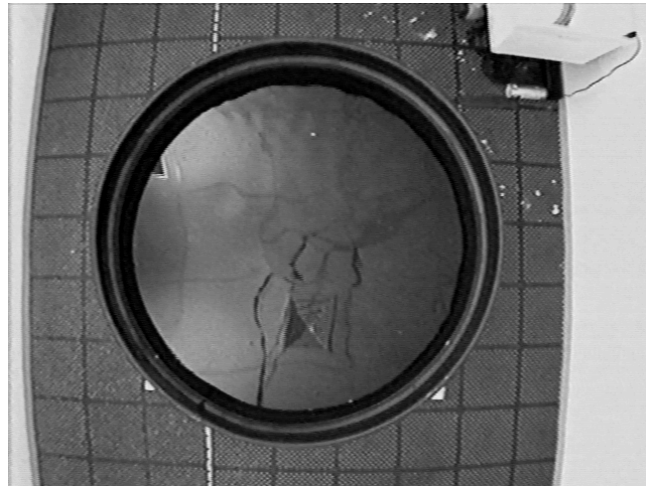


Figure 2.6. A well framed image of a water-maze. Zooming more than this would remove the border at the top and bottom of the image while zooming less would make the water-maze unnecessarily small.

Adjusting the lighting

ANY-maze works well in most lighting conditions but particular care needs to be taken when working with a water-maze as reflections from the water surface can cause problems with the tracking.

- ❑ If you are using a camera with a manual iris lens then adjust the iris so the picture has good contrast but isn't too bright – the image in Figure 2.6 is good in this respect.
- ❑ If you are using a water-maze then try to avoid using lights mounted directly over the maze. As can be seen in figure 2.6, the ceiling of the room reflects in the water and any lights will create strong, bright reflections that will interfere with the tracking.
- ❑ Often the best solution for a water-maze is to illuminate the apparatus using lights that point up and reflect off the ceiling.

If possible avoid using lights mounted directly over a water-maze as their reflections in the water can interfere with the tracking.

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Chapter 3

Performing a simple experiment

Introduction

This chapter is designed as a tutorial and will take you through the essential steps required to set up, run and analyse a simple water-maze experiment.

If you wish to, you can actually perform the steps in this tutorial as you go along using a training video included in ANY-maze (so you won't need any physical apparatus or animals). However, the tutorial includes lots of pictures and can be read independently, if that's more convenient.

At the end of the tutorial you should have a clear understanding of the overall structure of ANY-maze and of the general concepts used in the program.



The **New experiment** button offers a quick alternative to using the menu option.

Creating an experiment

ANY-maze is like Microsoft Word or Excel, in that you can't actually do anything very useful without first opening or creating a file. In fact, in ANY-maze each file represents an experiment, so the program tends to refer to experiments rather than files.

So, our first step will be to create a new experiment.

1. Start ANY-maze and, if necessary, log-on.
2. From the **File** menu select **New experiment**.

Setting up the protocol

What is a protocol?

Perhaps the single most important thing in ANY-maze is the *Protocol* as this is what tells the program how your experiment will be performed. For example, the protocol identifies:

- Which camera shows your apparatus (you might have more than one);
- What part of the image the camera shows is actually your apparatus;
- How you'd like the apparatus divided up, if at all. For example, into the open and closed arms of a plus-maze;
- How long tests should last;
- Etc, etc.

So, before you can perform an experiment you need to set up a protocol otherwise ANY-maze won't have any idea what it's supposed to be doing.

At first acquaintance setting up a protocol can seem rather a daunting task, but as you'll see during this tutorial, you don't actually have to do very much to create a basic protocol that contains enough information for you to start running tests. And, best of all, protocols can be saved and then

reused in future experiments - so in many experiments you won't need to set one up at all, you'll just load one you already have.

Of course in this tutorial we will be setting up a protocol, so the first step is to switch to the *Protocol* page. You do this simply by clicking the word **Protocol** in the ANY-maze *page bar* – see figure 3.1.

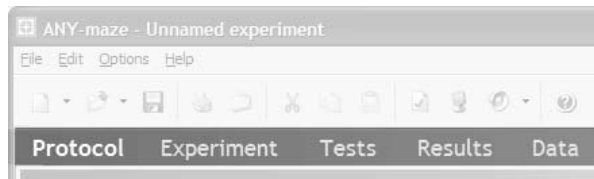


Figure 3.1. The ANY-maze page bar. Clicking a page title switches to that page.

The structure of a protocol

On the *Protocol* page you will see three panes, titled *Protocol*, *Settings* and *Image* – see figure 3.2.

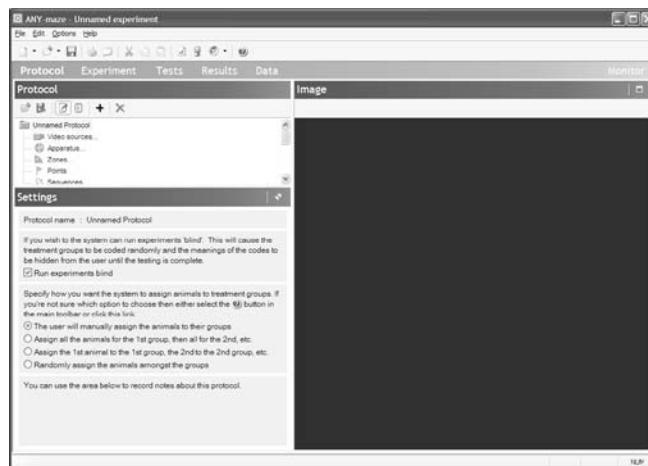


Figure 3.2. The ANY-maze Protocol page.

The *Protocol pane* displays a graphical representation of the protocol and, as can be seen in the figure 3.3, this shows how the protocol is divided into a number of different *elements*.

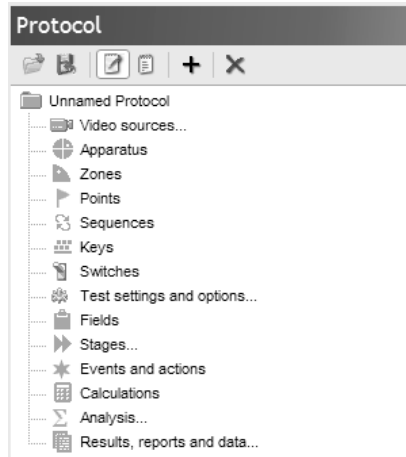


Figure 3.3. The protocol pane.

Although there are quite a lot of elements, many of them are optional so, for example, there's no need to include any *Points* in your protocol if you don't want to. In fact only four of the elements are compulsory.

The other two panes on the *Protocol page*, the *Settings and Image panes*, are used to enter or edit details of whichever element is current selected in the *Protocol pane*.

Naming the protocol

The first thing we need to do is name the protocol.

1. If it's not already selected, select the *root* element in the protocol list – this is the item at the very top, which will currently be called *Unnamed Protocol* (see figure 3.3).

2. The *Settings pane* will now show the settings for this element (see figure 3.4.) and the first item is the **Protocol name** - change it to Water-maze.
3. While we're here, you should also change the animal assignment option to **Assign all the 1st animal to the 1st group, the 2nd to the 2nd group, etc.** The reason you need to do this is explained later in this tutorial.

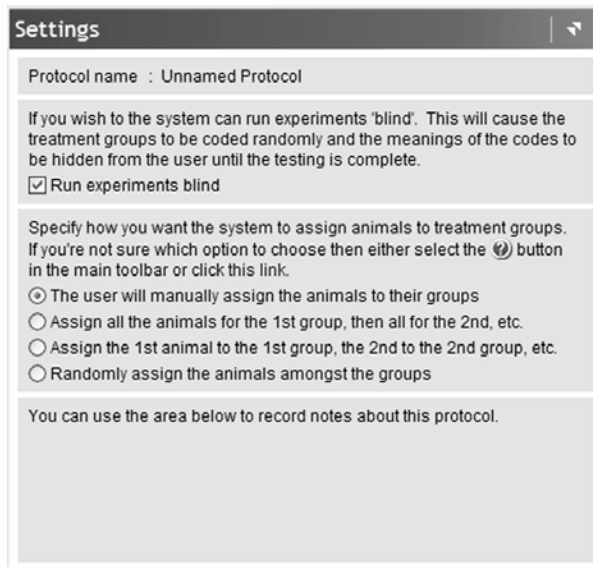



Figure 3.4. The settings pane showing the settings for the Protocol as a whole.

Adding a video source

Next we need to add a *Video source* to the protocol – this will specify where the video images of the apparatus are actually coming from.

1. Click the  button shown at the top of the *Protocol pane*.
2. Select **New video source** from the menu that appears.

3. A new video source will be added into the protocol and will appear in the *protocol pane* as in figure 3.5.

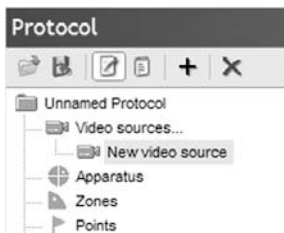


Figure 3.5. The Protocol pane after adding a new video source

4. The *Settings* pane will also change and will show the settings for the video source, as in figure 3.6.

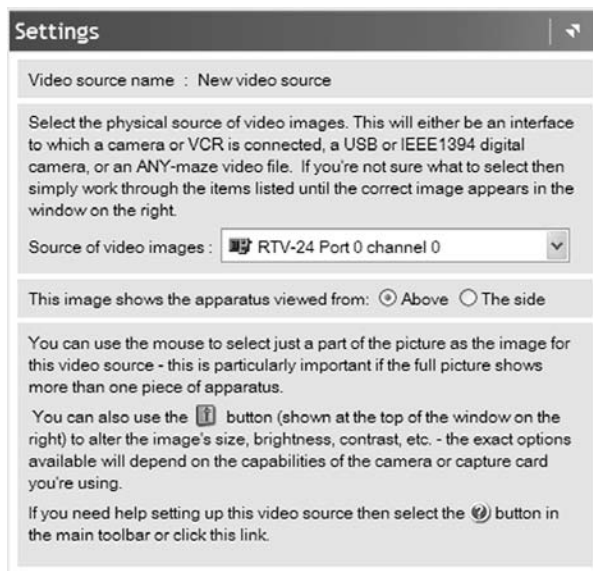


Figure 3.6. The Settings pane showing settings for the new video source.


5. The *Image pane* will now show either an image from a camera, if you have one attached to your computer, or an example image if you don't.
6. As mentioned above, the video source identifies where the video images are coming from. In this tutorial we're going to use an example video that's provided with ANY-maze, so we need to select this video as the *Source of video images*. To do this simply open the **Source of video images** list and choose the entry for **Water-maze example video**. (If this entry isn't present then choose **Water-maze example image** instead.)
7. The video source will automatically have been given a name of *New video source*, change this to `Water-maze camera`. You can do this by simply overtyping the name shown in the *Settings pane*.
8. That's all we're going to do with our video source.

Adding apparatus

The video source tells ANY-maze where video pictures are coming from, but it doesn't tell it anything about the apparatus in the pictures, that's the job of another protocol element – *Apparatus*.

The apparatus element tells ANY-maze what parts of the image are the apparatus, as well as allowing you to divide the apparatus into different areas that you can subsequently designate as *Zones*.

So we need to add an apparatus element for our water-maze.

1. Click the  button shown at the top of the *Protocol pane*.
2. Select **New Apparatus** from the menu that appears.
3. The new apparatus will be added into the protocol and will appear in the protocol pane as in figure 3.7.

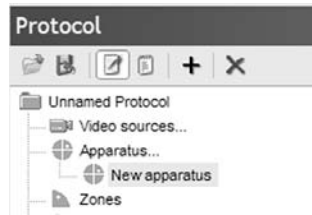


Figure 3.7. The Protocol pane showing the new Apparatus.

4. The *Settings* pane will change to show the settings for the apparatus, as in figure 3.8.



Figure 3.8. The Settings pane showing the settings for the new apparatus.

5. The *Image* pane will also change and will show the image from the *Water-maze camera video source* that we added in the previous step.

6. You now need to draw what ANY-maze calls the *Apparatus map*. This map defines the boundaries of the apparatus and, optionally, divides it into different areas that interest you – see figure 3.9.

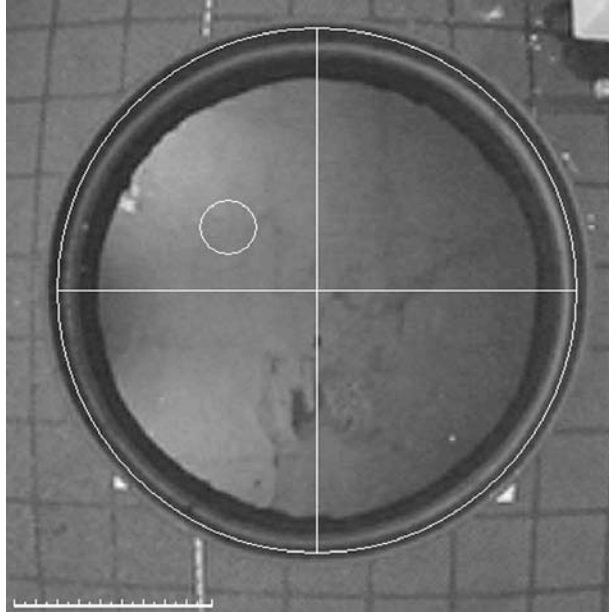







Figure 3.9. A simple apparatus map for a water-maze. This map defines the borders of the maze and divides it into four quadrants. The position of an island is also marked.

7. The apparatus map is drawn using various drawing tools shown at the top of the *Image pane*. These are:
- | | | |
|---|----------------|---|
|  | Selection tool | Selects an element of the drawing so you can move, resize or delete it. |
|  | Line tool | Draws a straight line from the point where you press the left mouse button to the point where you release it. |

	Rectangle tool	Draws a rectangle with one vertex at the point where you click the left mouse button and the diagonally opposite vertex at the point where you release it.
	Ellipse tool	Draws an ellipse starting from the point where you click the left mouse button and ending at the point where you release it.
	Multi-line tool	Draws multiple joined line segments. A new segment starts at each mouse click. A double click ends the drawing.

If you've previously used a drawing program, such as the drawing functions in Microsoft Word, then you should find that these tools work in a familiar and expected way.

8. You should now use the *Ellipse tool* to draw the border of the apparatus, and then use the *Line tool* to divide the apparatus into quadrants. You'll find that the pole points of the ellipse and the ends of the lines attract each other which makes it very easy to join them together. Finally add a small ellipse to represent the island. (Don't worry too much about where it is). When you finish your map should look like the one in figure 3.9.
9. You may have noticed in figure 3.9 there's something that looks like a scale in the bottom left corner of the image. This is what's called the *ruler line* and is used to set the scale of your image so that results can be reported in real-world units such as metres and metres-per-second.

Setting the scale is a two-step process:

- o First use the mouse to drag the ends of the *ruler line* so it is positioned along something of a known length in your image. In figure 3.10 it's been positioned across the diameter of the water-maze, as this is a distance we'll probably know.
- o Enter the actual length of the object the *ruler line* is positioned on into the field titled **The length of the ruler line is** in the

Settings pane. In our case the tank is 120cm across so you'd enter 1200 (note that the distance is specified in millimetres).

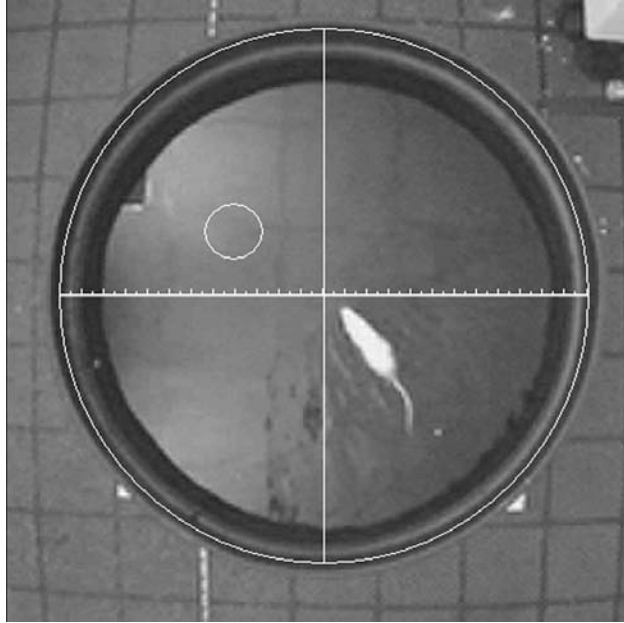



Figure 3.10. The apparatus map with the ruler line positioned across the diameter of the water-maze.

10. Finally you should change the name of the apparatus to Water-maze. You can do this by simply overtyping the name shown in the *Settings pane*.

Adding some zones

Although the *apparatus map* divides the apparatus into different areas it doesn't actually identify them individually, this is the job of the *Zones* element.

Unlike the *Video source* and *Apparatus* elements, *Zones* are optional – it might be that you simply don't care about the animal's behaviour in different areas of the apparatus. However, we'll add some zones to our protocol so you get the idea about how they work.

1. Click the  button shown at the top of the *Protocol pane*.
2. Select **New Zone** from the menu that appears.
3. In the *Settings pane* type in a name for the zone, let's call it North West – see figure 3.11.

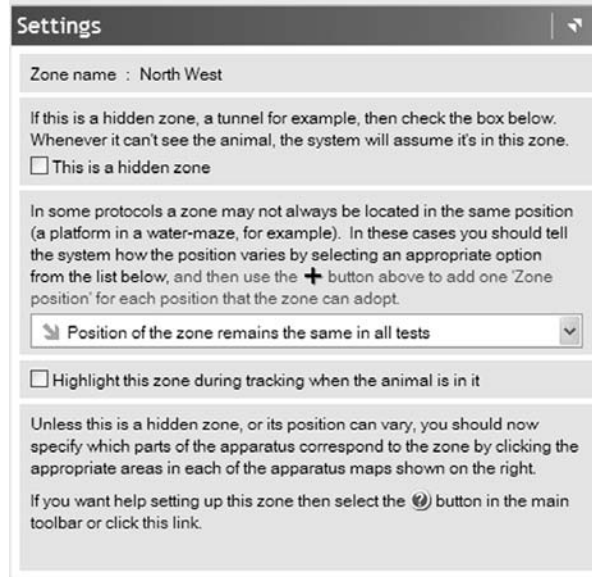


Figure 3.11. The settings pane for Zones.

4. In the *Image pane* click the area, or areas, of the *apparatus map* that constitute the zone – see figure 3.12.

Note that a single zone can consist of as many areas of the map as you like and these areas need not be contiguous. For example, in the

plus maze the open arms zone would consist of two non-contiguous areas. Also the same area can be part of more than one zone.

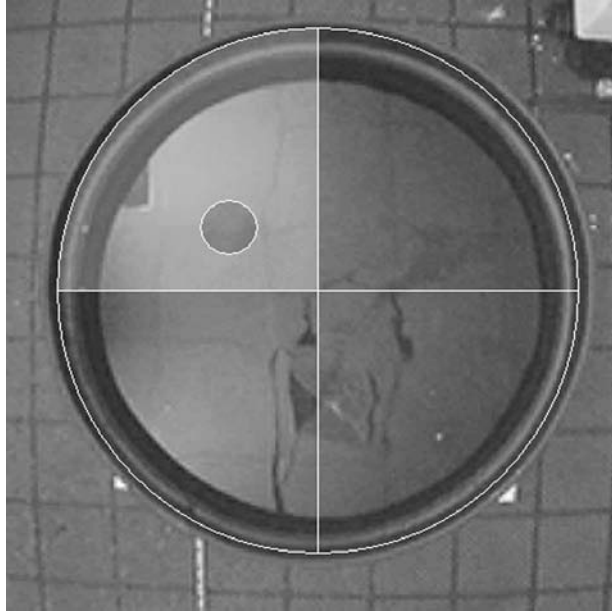


Figure 3.12. The area of the water-maze that constitutes the North West zone. (On screen the area is highlighted in colour making it clearer).

Having created the *North West* zone you can go on to create a *North East* zone – the process is just the same. If you like, you can add *South West*, *South East* and *Island* zones too.

Note that here we're seeing a new feature of the protocol – multiple elements, see figure 3.13. In fact, you can also have multiple *Video sources* and multiple *Apparatus*, but these features are beyond the scope of this tutorial.

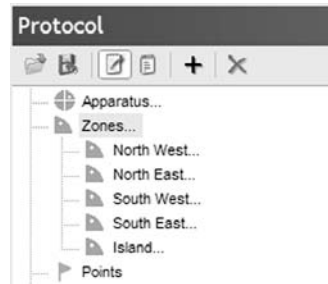


Figure 3.13. A water-maze protocol containing five zones.

So far we have been working through the protocol elements in the order they appear in the protocol list (see figure 3.3). However, we're now going to skip the *Points*, *Keys*, *Sequences* and *Switches* elements as these are all optional and we don't need any of them in our water-maze. So the next element we'll be considering is the *Test settings and options*.

Setting the animal colour

ANY-maze doesn't require you to make any special settings in order for it to track your animals. However there is one thing it does need to know: the colour of the animals; or to be precise whether the animals are lighter or darker than the apparatus background. This is defined in the protocol's *Test settings and options*.

The *Test settings and options* are different to the other protocol elements we've used so far, as they're already present in the protocol - so there's no need to add them.

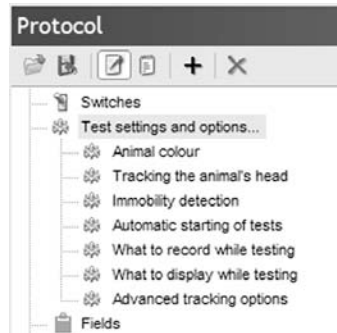


Figure 3.14. The Test settings and options protocol element.

To set the animal colour do the following:

1. Click the **Test settings and options** element in the protocol. It will open showing a series of sub elements – see figure 3.14.
2. Click the **Animal colour** sub element.
3. In the settings pane click the option labelled **The animals are lighter than the apparatus background** (the animals in the video we're using are white) – see figure 3.15.

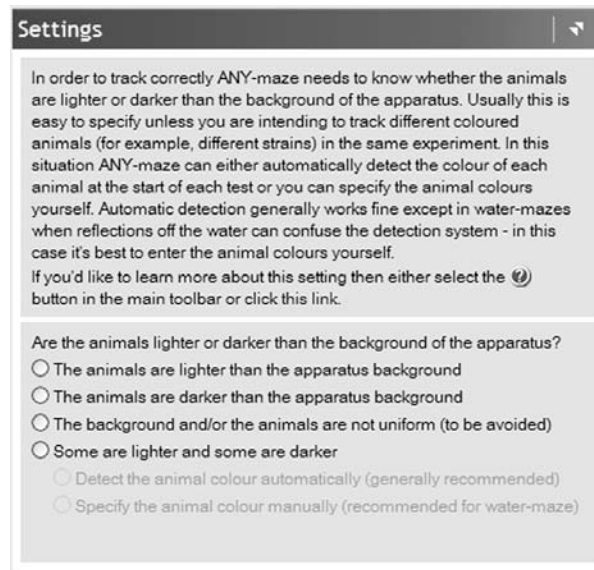


Figure 3.15. The Animal colour settings.

As you can see in figure 3.14, there are a range of other *Test settings and options* that we could use, but we don't actually need any of them in our protocol.

Settings test duration

We're nearly done. There's just one step left to complete our basic protocol and that's to specify how long the tests in our water-maze will last. This is done using the protocol's *Stage* element.

A *stage* in ANY-maze represents a series of repeated *trials*, each one of an equal duration. Each stage can include up to 50 *trials* and you can include up to 16 different stages.

In a water-maze we would probably have two stages called something like "Training" and "Probe". The Training stage would probably include a few

trials, while the Probe stage would probably include just one. However, to keep things simple we're going to set up a single stage with a single trial.

In fact, a protocol must always include at least one stage, and so ANY-maze automatically added one to our protocol when we created the experiment file. Therefore all we need to do is edit this stage to suit our requirements:

1. Click the **Stages** element in the *Protocol pane*.
2. The element will open to show the **First stage**, which was put there automatically by ANY-maze – click this item.

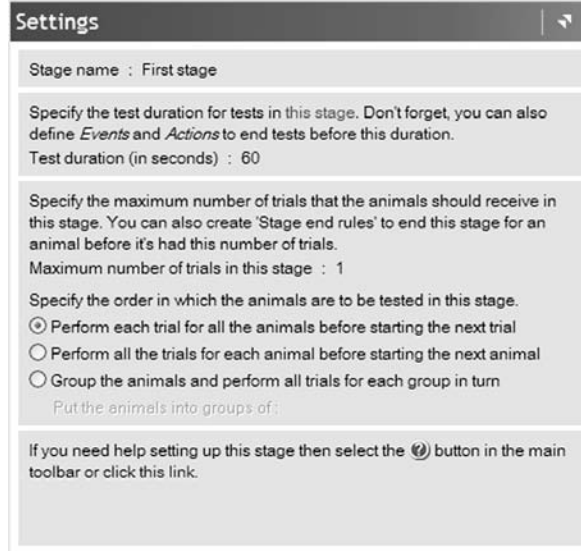



Figure 3.16. The settings for the “First stage” that ANY-maze automatically includes in all protocols.

3. The *Settings pane* will now show the settings for this default *First Stage* – see figure 3.16.
4. As you can see this stage is called *First stage* and consists of a single trial that lasts for 60 seconds. Although you could change any of

these settings, we'll just leave them as they are, except for the test duration, which you should change to 30 seconds.

Saving the protocol

We're done! The protocol is now complete and we can use it to run an experiment. However, before going on to do this, we'll save the protocol so we can use it again in the future.

1. Click the  button shown at the top of the *Protocol Pane*.
2. The *Save Protocol window* will open – see figure 3.17. Enter a name for the protocol and choose where you want to save it, then click **OK**.

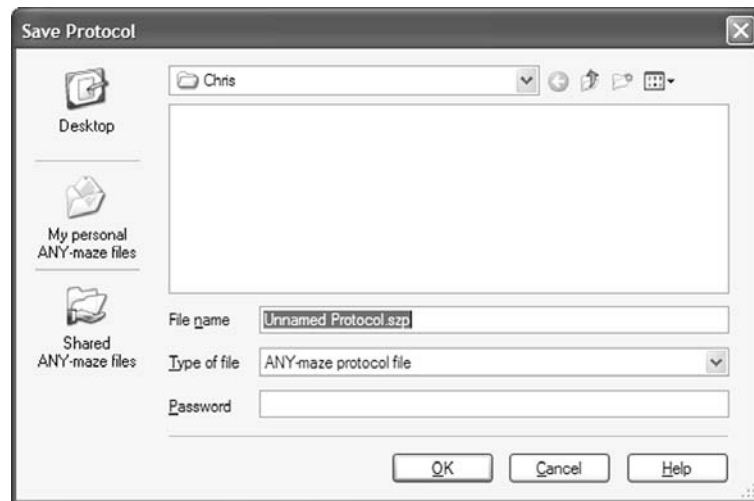


Figure 3.17. The *Save Protocol* window.

It's a good idea to save protocols either to your *Personal ANY-maze folder*, which will be selected by default, or if you want other users to be able to use the protocol as well, to the *Shared ANY-maze folder*. The reason this is recommended is because ANY-maze will automatically look

in these folders for protocols and this will make it easier to create new experiments that use them.

A final word

If you work with water-mazes you may be wondering whether our protocol will automatically end tests when an animal finds the island. The answer to this is that it *could*, but to do this we would need to add some more elements to the protocol. Therefore, to prevent this tutorial from becoming too complex, this step has been omitted.

If you're curious about how this would be done, refer to the *Events and Actions* section in chapter 4.

Setting up the experiment

So far we've set up the protocol for our water-maze. As you'll appreciate this protocol could be used in many different water-maze experiment as it simply defines the physical maze, its zones and the duration of the tests, and doesn't include any information about the animals or treatments we intend to use. This information is part of the *experiment*.

So, there's a division here between the protocol, which defines how an experiment will be performed, and the experiment, which defines what tests we'll actually be performing – this is why it's possible to save a protocol and use it repeatedly in different experiments.

Clearly then, we need to tell ANY-maze about the experiment we're about to perform. To keep things simple we're going to set up our experiment to test just 12 animals divided into two groups – one group will be treated with Compound X and the other with Saline. This information is entered on the *Experiment* page:

1. Switch to the *Experiment* page by clicking the word **Experiment** in the ANY-maze *page bar*.

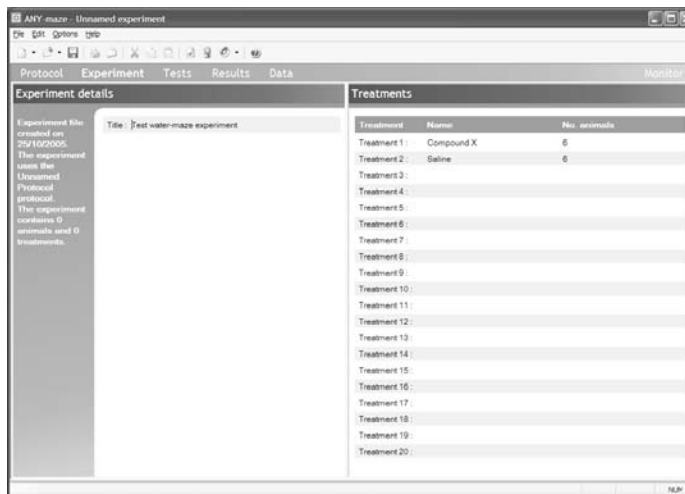


Figure 3.18. The Experiment page.

2. The *Experiment page* is divided into two panes, on the left is the *Experiment Details pane* and on the right the *Treatments pane* – see figure 3.18.
3. The *Experiment details pane* is very simple and consists of just two things: The experiment title and an area where you can record notes about the experiment. In fact both entries are optional, but let's enter a title anyhow, type in: `Test water-maze experiment`.
4. As the name implies, the *Treatments pane* is used to enter the different treatment groups in the experiment. For each group you enter a name and the number of animals it contains – i.e. its N. So, for our experiment enter: `Compound X`, with an N of 6 and `Saline`, also with an N of 6.

Note that you don't *have* to divide animals into groups; you could just enter a single *treatment* called, say, `Animals`.

And that's it; our experiment is now ready to be performed.

Running the tests

As you will probably have guessed, the tests are performed using the *Tests* page. Like the *Experiment* page, this page is divided into two panes, the *Experiment management pane* and the *Apparatus pane* – see figure 3.19.

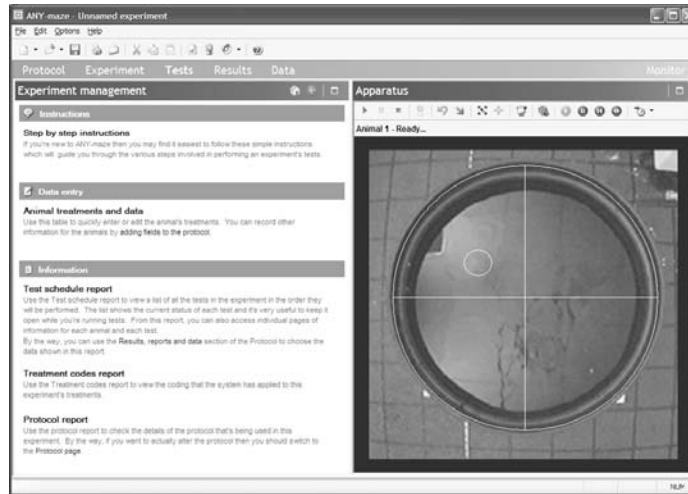





Figure 3.19. The Test page

As the names imply, the *Experiment management pane* is used to manage the experiment as a whole, while the *Apparatus pane* is used to perform the individual tests in the apparatus.

So, let's proceed directly to running a test.

1. While you're learning how to use ANY-maze it's a good idea to use the system's *Step-by-Step instructions* as these will guide you through the process of performing the tests. To open these instructions click the **Step-by-Step instructions** link shown at the top of the *Experiment management* home page.
2. As the instructions will tell you, you should now *Prepare for the test of animal number 1*. Normally this would mean getting the animal from its cage and so forth, but as we're just using a video of a test

there's not much preparation we need to perform – we just need to rewind the video to the start. To do this, click the  button at the top of the *Tests pane*.

3. Now we're ready to start the test. This is a two stage process:
 - a. First click the  button, which will cause the test status to change to *Waiting to start*.
 - b. Next you would normally walk up to the apparatus, place the animal in it and then walk away. As ANY-maze sees you do this it would start the test. Of course you can't do this because we're using a video, but to see it happen just click the  button, which will start the video playing.
4. When the test starts ANY-maze will indicate the position of the animal with an orange dot.
5. The test will run for 30 seconds, the duration we specified in the protocol, and then stop – happily this is just when the animal finds the island!
6. ANY-maze will save the results of the test and will then be ready to test animal number 2.

This experiment uses the ANY-maze auto-start feature, but you can also start tests manually if you prefer.

And that's the testing process. If you like you can run through all 12 animals at which point the test status will change to *Testing complete*.

Managing the experiment

As you may have noticed, the animals in the experiment are given sequential numbers and are simply tested in order. But that being the case how do you know which treatment each animal should receive - Compound X or Saline? The answer is to look at the *Test schedule report*, which tells you this.

The *Test schedule report* is one of a range of different reports that can be accessed in the *Experiment management pane*.

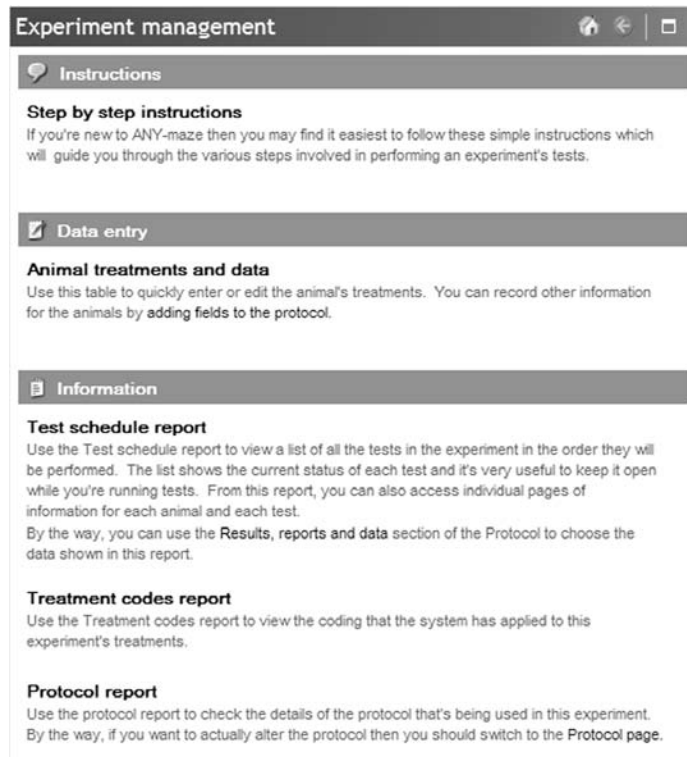

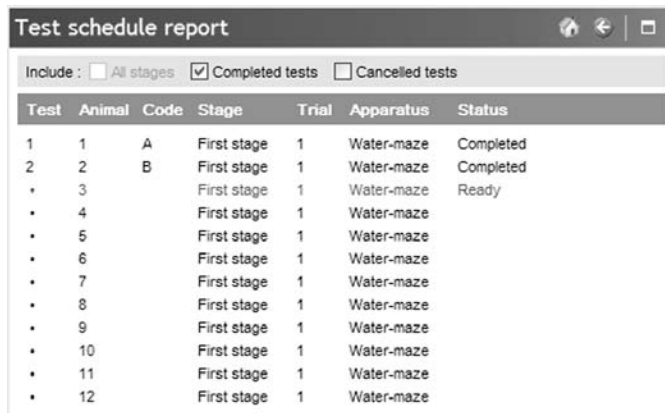


Figure 3.20. The Experiment management “home page”.


1. If you followed the above instructions then the *Experiment management pane* will currently be showing the *Step-by-Step Instructions*. To return to the *Experiment management home page*, simply click the  button shown in the pane’s title bar.
2. Then click the link titled **Test schedule report**.
3. The *Test schedule report* will open - see figure 3.21. This report shows the order of the tests that will be performed in the experiment. As you’d expect, in our experiment we will simply test animals 1 to 12 in sequence.



Test	Animal	Code	Stage	Trial	Apparatus	Status
1	1	A	First stage	1	Water-maze	Completed
2	2	B	First stage	1	Water-maze	Completed
•	3		First stage	1	Water-maze	Ready
•	4		First stage	1	Water-maze	
•	5		First stage	1	Water-maze	
•	6		First stage	1	Water-maze	
•	7		First stage	1	Water-maze	
•	8		First stage	1	Water-maze	
•	9		First stage	1	Water-maze	
•	10		First stage	1	Water-maze	
•	11		First stage	1	Water-maze	
•	12		First stage	1	Water-maze	

Figure 3.21. The Test schedule report

You'll notice that next to each animal is a code of either A or B – this denotes the treatment that the animal is to receive. But two obvious questions arise, which treatment is A and which is B and how does ANY-maze know which treatment to give to each animal?

The answer to the first question can be found on another of the experiment management reports, the *Treatment codes report*. If you want to take a look at it now, simply click the  (back) button, which will return to the *Experiment management home page* and then click the **Treatment codes report** link.



Treatment	Code
Compound X	A
Saline	B

Figure 3.22. The Treatment codes report

You may be wondering why the treatments are given codes at all. This is to facilitate blind experiments. In this case someone other than the experimenter would use the treatment codes report to see what coding has

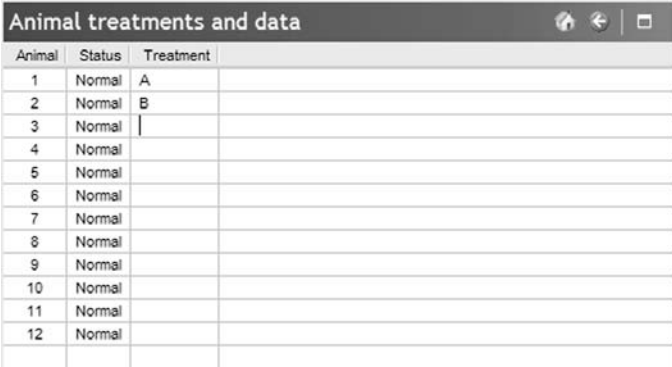
been applied to the treatments and then label the treatments with their codes. The experimenter would then apply the appropriate coded treatment to each animal without actually knowing which treatment is which.

- ❑ If you wish to, you can alter the *Test schedule report* so it shows the actual treatment names rather than their codes.

The answer to the second question about treatments - how ANY-maze knows which treatment to apply to each animal - is that we told it how to do this as part of the protocol.

You may recall that when we set up the protocol, you chose an option to *Assign the 1st animal to the 1st group, the 2nd to the 2nd group, etc.* – and this is how the animals have been assigned.

Of course, there are other ways to assign the animals to the groups, for example randomly, and there's also an option that allows you to do this manually. In the latter case ANY-maze won't automatically assign the animals to any treatment group and you'll need to do this yourself using the *Animal treatments and data page* - which is something else you can access from the *Experiment management home page*.



Animal	Status	Treatment
1	Normal	A
2	Normal	B
3	Normal	
4	Normal	
5	Normal	
6	Normal	
7	Normal	
8	Normal	
9	Normal	
10	Normal	
11	Normal	
12	Normal	

Figure 3.23. The *Animal treatments and data page*

As you'd expect, there are other options related to experiment management, for example you can skip over a test, delete an animal, change a treatment, etc., but these are beyond the scope of this tutorial.

Looking at test results

You will doubtless have noticed that so far we haven't seen any results for our tests – nothing to tell us how far the animal swam, how long it took to find the island, etc.

We'll now finish off this tutorial by exploring the various options available to view, analyse and transfer test results.

Looking at results of individual tests

Returning to the *Test schedule report* – see figure 3.21 – you will find that the test numbers shown in the left-most column are in fact links and that clicking one takes you to the test's *Test details report* – try it on a test you've performed.

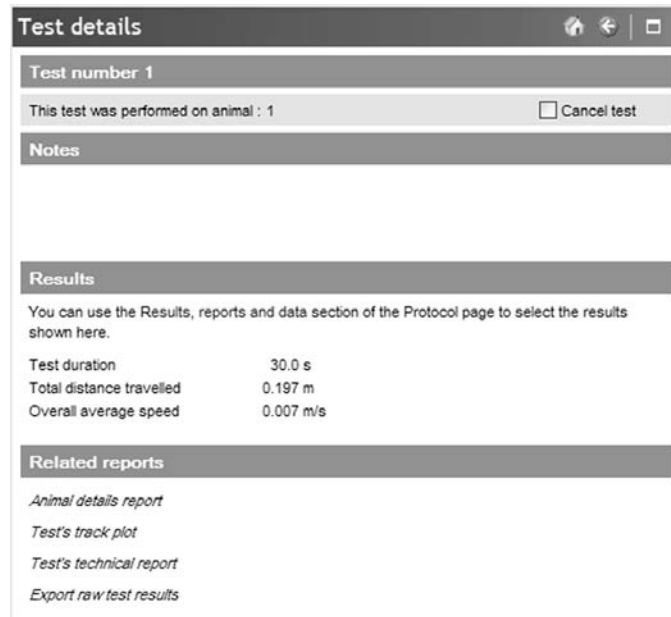


Figure 3.24. The Test details report

As the name implies, the *Test details report* shows information relating to an individual test and included in the report is a section showing the test results.

As you can see in figure 3.24 there aren't many results shown - for example, there's nothing relating to the time the animal spent in each of the zones we included in the protocol. This is simply because the report defaults to show a minimal number of *measures*, but you can easily alter the report's definition (which is part of the protocol) to include whatever measures you like.

Working with results of multiple tests

Although the result for individual tests may be of interest you will probably want to look at your results collated into groups and most likely you'll want to analyse them using some statistical tests.

As the experiment you've created in this tutorial uses a video of a single test, the results of all the tests will, necessarily, be the same. Clearly this will make it rather meaningless to try to analyse differences between the treatment groups, and so before proceeding any further you need to save your experiment (if you want to) and load an example experiment that includes more data.

1. From the **File** menu select the **Open an example experiment** option.
2. A sub menu will appear. Select the first option – **Example water-maze experiment**.
3. A message will be displayed asking whether you'd like to save the experiment you currently have open, if you would like to then click **Yes** – the *Save experiment window* will open for you to choose the name and folder to save the file to.
4. Now, with the example experiment open, switch to the *Results page* by clicking the word **Results** in the ANY-maze *page bar*.

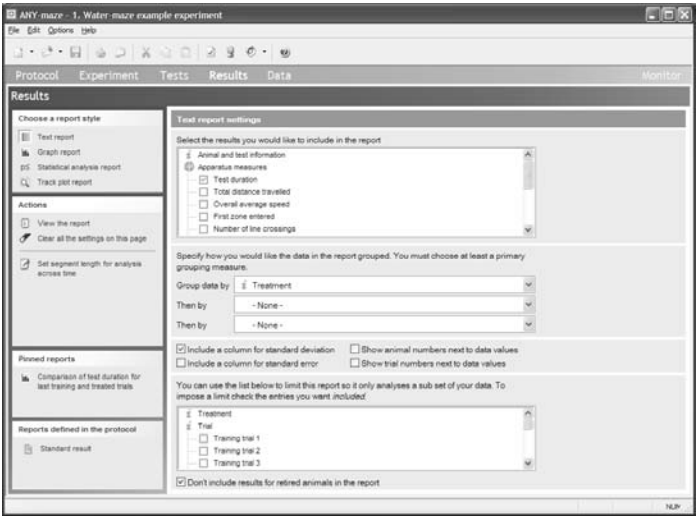


Figure 3.25. The Results page

To get a quick idea about how the results page works, click the **View the report** link in the **Actions** panel on the left side of the page. A *Text report* will appear – see figure 3.26

Test duration (s)				
Treatment	N	Mean	SD (note 1)	Data (notes 2, 3)
Saline	6	9.40	±6.02	19.0, 8.5, 11.3, 3.8, 11.4, 2.4
Compound X 1.0 mg/kg	5	18.74	±11.51	3.1, 30.7, 24.3, 10.4, 25.2
Compound X 5.0 mg/kg	5	33.46	±16.19	43.5, 35.7, 51.9, 26.2, 10.0
Compound X 10.0 mg/kg	5	83.80	±31.32	118.5, 110.3, 86.0, 53.2, 51.0

Notes:

- SD = Standard deviation.
- The data analysed has been limited in the following way: Trial = Treated trial.
- Animals which are marked as retired have been excluded from this report.

Figure 3.26. Example of a text report

As can be seen, this report shows results of the *Test duration* measure collated by *Treatment*. For each treatment group the N, mean and standard deviation are shown together with the data for the individual animals.

If you study the data you'll notice that increasing doses of Compound X seem to be increasing the Test duration, but a graph of the same data would probably make this clearer. Click the **Graph report** link in the *Choose a report style* panel on the left of the page – a graph will appear.

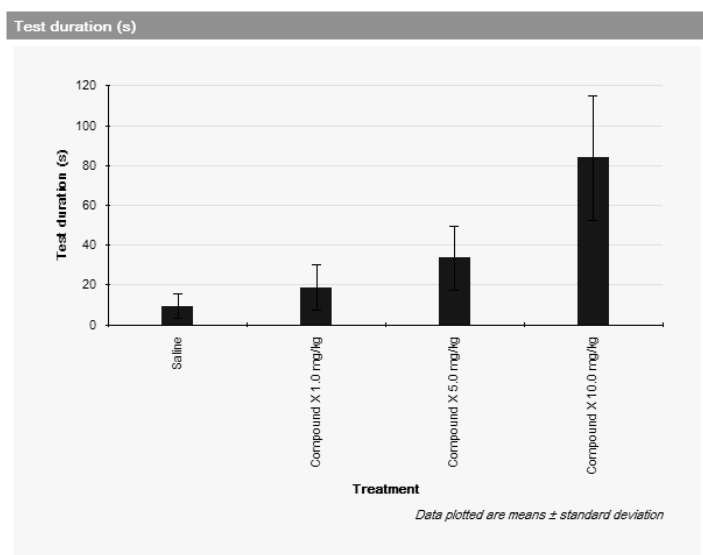


Figure 1. Test duration. The data analysed has been limited in the following way: Trial = Treated trial. Animals marked as retired have also been excluded from this graph.

Figure 3.27. A graph showing the same data as figure 3.26

It's now clear that Compound X is increasing the Test duration, but is this effect significant? Click the **Statistical analysis report** link in the *Choose a report style* panel to find out.

Test duration (s)				
Treatment	N	Mean	SD (note 1)	Data (notes 2, 3)
Saline	6	9.40	±6.02	19.0, 8.5, 11.3, 3.8, 11.4, 2.4
Compound X 1.0 mg/kg	5	18.74	±11.51	3.1, 30.7, 24.3, 10.4, 25.2
Compound X 5.0 mg/kg	5	33.46	±16.19	43.5, 35.7, 51.9, 26.2, 10.0
Compound X 10.0 mg/kg	5	83.80	±31.32	118.5, 110.3, 86.0, 53.2, 51.0
ANOVA (notes 4, 5): $F_{(3,17)} = 17.1234$ $p = 0.000$				
Dunnett's test (notes 5, 6)				
Saline vs. Compound X 1.0 mg/kg			$q_{(17,1)} = 0.8436$	$p = 0.411$
Saline vs. Compound X 5.0 mg/kg			$q_{(17,2)} = 2.1732$	$p = 0.080$
Saline vs. Compound X 10.0 mg/kg			$q_{(17,3)} = 6.7200$	$p = 0.000$
Notes:				
1. SD = Standard deviation.				
2. The data analysed has been limited in the following way: Trial = Treated trial.				
3. Animals which are marked as retired have been excluded from this report.				
4. One-way ANOVA, fixed factor.				
5. $p \leq 0.05$ is highlighted in red.				
6. Dunnett's step-down test. [Dunnett and Tamhane. Step-down multiple tests for comparing treatments with a control in unbalanced one-way layouts, Statistics in Medicine, 10 (1991): 939-947].				

Figure 3.28. Statistical analysis of the data shown in Figure 3.27.

So we can see that the Compound X is causing a significant effect at a dose of 10 mg/kg.

But here we've been looking at just the one result, *Test duration*, and the data has been collated by *Treatment* and the statistical test used was an ANOVA followed by *Dunnett's post hoc test for comparisons to a control* – so what if that's not what you want?

These parameters, and more, were all set on the original Results page we started at. To go back to it now click the link labelled **Change the report settings** in the *Actions* panel.

As the currently selected report style is *Statistical analysis report*, the options you will see are related to analysis of the data, see figure 3.29.

Statistical analysis report settings

Select the dependent variables you wish to analyse.

☒ Animal and test information

☒ Apparatus measures

☒ Test duration

☐ Total distance travelled

☐ Overall average speed

☐ First zone entered

Select the 1st independent variable:

Optionally select a 2nd independent variable:

Select the type of statistical tests to use: ☒ Parametric ☐ Non-parametric

Optionally select a post-hoc test to use:

Select the control group to compare to:

☒ Include a column for standard deviation
 ☐ Show animal numbers next to data values

☐ Include a column for standard error
 ☐ Show trial numbers next to data values

You can use the list below to limit this report so it only analyses a sub set of your data. To impose a limit check the entries you want *included*.

☒ Treatment

☒ Trial

☐ Training trial 1

☐ Training trial 2

☐ Training trial 3

☒ Don't include results for retired animals in the report

Figure 3.29. The statistical analysis report options

Here you can set:

- The dependent variable to analyse
- The independent variables
- Whether to use parametric or non-parametric statistical tests.
- The tests to use.
- The data that should be shown on the report.
- Limits - which will cause just a subset of the experiment's results to be analysed.

Explaining all these options is beyond the scope of this tutorial, but you'll probably find they work much as you'd expect anyhow.

If you change the report style, for example, to a graph report, then you will be offered similar options, but specific to graphs, so for example, you'll be able to choose the graph style (line, column, scatter), error bars, etc.

Transferring results to other programs

The results reports described in the previous section are designed to provide a clear format for reviewing collated results of your tests. However, their format is not suitable for transferring data to other programs such as Excel or SPSS – to do this ANY-maze includes the *Data page*.

To access the Data page simply click the word **Data** in the ANY-maze *page bar*.

As can be seen in figure 3.30, the *Data page* shows results in a tabular format that's ideal for transfer to spreadsheet, statistics or database programs.

- ❑ By clicking the **Select data to show** link in the *Actions* panel on the left of the page, you can choose which tests (i.e. rows) and which measures (i.e. columns) are included in the spreadsheet.
- ❑ You can use the mouse to select rows, columns or cells and you can copy these to the clipboard and paste them straight into other programs.
- ❑ You can also save the entire spreadsheet, or a selection, to a file in a variety of formats suitable for almost all analysis programs.

Test	Animal	Treatment	Code	Stage	Trial	Reason ended	Time	Date	Distance	Island - latency to first entry
1	1	Saline	A	Training	1	Test duration	18:61	10/02/2003	31.204m	120.0s
2	2	Compound X 6.0 mg/kg	C	Training	1	Test duration	18:63	10/02/2003	26.040m	120.0s
3	1	Saline	A	Training	2	Island entry	18:66	10/02/2003	2.076m	26.8s
4	2	Compound X 6.0 mg/kg	C	Training	2	Island entry	18:67	10/02/2003	0.320m	2.6s
5	1	Saline	A	Training	3	Island entry	18:67	10/02/2003	2.901m	11.6s
6	2	Compound X 6.0 mg/kg	C	Training	3	Island entry	18:69	10/02/2003	6.086m	26.1s
7	3	Compound X 1.0 mg/kg	B	Training	1	Island entry	19:01	10/02/2003	13.161m	62.6s
8	3	Compound X 1.0 mg/kg	B	Training	2	Island entry	19:02	10/02/2003	1.830m	7.5s
9	4	Compound X 10.0 mg/kg	D	Training	1	Island entry	19:05	10/02/2003	8.317m	43.8s
10	4	Compound X 10.0 mg/kg	D	Training	2	Island entry	19:06	10/02/2003	21.732m	91.2s
11	3	Compound X 1.0 mg/kg	B	Training	3	Test duration	19:08	10/02/2003	21.649m	120.0s
12	4	Compound X 10.0 mg/kg	D	Training	3	Island entry	19:10	10/02/2003	2.432m	10.5s
13	3	Compound X 1.0 mg/kg	B	Training	4	Island entry	19:11	10/02/2003	3.633m	17.0s
14	4	Compound X 10.0 mg/kg	D	Training	4	Island entry	19:12	10/02/2003	2.332m	7.9s
15	3	Compound X 1.0 mg/kg	B	Training	5	Island entry	19:12	10/02/2003	1.229m	6.1s
16	5	Saline	A	Training	1	Island entry	19:16	10/02/2003	7.151m	31.4s
17	6	Compound X 10.0 mg/kg	D	Training	1	Island entry	19:17	10/02/2003	12.886m	43.9s
18	5	Saline	A	Training	2	Island entry	19:20	10/02/2003	6.221m	17.7s
19	6	Compound X 10.0 mg/kg	D	Training	2	Island entry	19:21	10/02/2003	6.246m	18.7s
20	5	Saline	A	Training	3	Island entry	19:22	10/02/2003	3.862m	14.2s
21	6	Compound X 10.0 mg/kg	D	Training	3	Island entry	19:22	10/02/2003	2.331m	11.4s
22	7	Compound X 1.0 mg/kg	B	Training	1	Island entry	19:23	10/02/2003	11.269m	41.6s
23	8	Compound X 6.0 mg/kg	C	Training	1	Island entry	19:24	10/02/2003	6.877m	23.3s
24	7	Compound X 1.0 mg/kg	B	Training	2	Island entry	19:24	10/02/2003	3.123m	13.7s
25	8	Compound X 6.0 mg/kg	C	Training	2	Island entry	19:28	10/02/2003	13.625m	44.3s
26	7	Compound X 1.0 mg/kg	B	Training	3	Island entry	19:32	10/02/2003	2.706m	9.9s
27	8	Compound X 6.0 mg/kg	C	Training	3	Island entry	19:33	10/02/2003	1.164m	5.9s
28	8	Compound X 6.0 mg/kg	C	Training	4	Island entry	19:33	10/02/2003	1.131m	6.7s
29	9	Saline	A	Training	1	Island entry	19:48	10/02/2003	8.489m	32.6s
30	10	Compound X 10.0 mg/kg	D	Training	1	Island entry	19:48	10/02/2003	16.898m	59.5s
31	9	Saline	A	Training	2	Island entry	19:51	10/02/2003	7.708m	29.6s

Figure 3.30. The Data page

Summary

That concludes this tutorial. To summarise what you have learnt:

- The protocol is fundamental to ANY-maze as it tells the program how an experiment should be performed.
- The protocol is divided into different elements.
- A few elements, such as Video sources, Apparatus and Stages are compulsory but most of them are optional and need only be included if you require their features in your apparatus.
- Protocols can be saved and reused in multiple experiments.
- Setting up an experiment consists of designing, or more normally loading, a protocol, and adding some animals, possibly divided into different treatment groups.
- Testing is performed on the Tests page and the Step-by-step instructions will guide you through the process.

- Alternatively you can simply use the Test schedule report to see what to do next.
- You can use the various experiment management reports and tables to manage all the details of the animals and tests in an experiment.
- Results for individual tests can be viewed in the Test details reports.
- Collated results of multiple tests can be viewed, plotted and analysed using the Results page.
- The Data page shows the results in a spreadsheet format suitable for transfer to other programs for further analysis.

You should now have a clear understanding of the structure of ANY-maze and how the program works and, with the assistance of the on-line help, you hopefully now feel confident enough to design and run your own experiments. And remember, the ANY-maze technical support staff are always on hand to help you with any questions you may have, no matter how minor.

Your Distributor is:
Braintree Scientific, Inc.
PO Box 850498, Braintree, MA 02185
781-917-9526
Email: Info@braintreesci.com
Web: www.braintreesci.com

Chapter 4

Protocols elements in detail

Introduction

ANY-maze protocols are at the heart of the system – it's through them that the program gains its power and flexibility. Clearly therefore, a good understanding of protocols is required if you're to get the most from the software.

This chapter will describe each element of the protocol in more detail with particular emphasis on practical example of how the features of an element can be used. These descriptions are not intended to serve as a step-by-step guide to setting up a protocol (you'll find this in the on-line help), but rather to explain what each of the elements is for.

This chapter assumes you have read the preceding chapter and that you are comfortable with the overall concept of the protocol and its structure.

The root protocol element

At the root of all protocols is a single *Protocol* element. In fact this element is very simple and, apart from providing a name for the protocol, is only really used to specify whether experiments are to be run blind and how animals are to be assigned to treatment groups. In our experience

most people prefer to manually assign animals to their treatments using the *Animals treatments and data page* in the *Experiment management* pane and therefore this is the option that's chosen by default. As these options are quite self-explanatory they're not described further here.

Video sources

A video source provides a flexible method to define and adjust the video image of a piece of apparatus.

Your goal when setting up a video source should be to finish with an image that has good contrast and shows a single piece of apparatus loosely framed within the picture.

Adjusting the video picture

Apart from video sources that show images from a digital video file, all sources include options to alter such things as brightness and contrast, although the exact functions available will depend on the camera or digitiser that you are using.

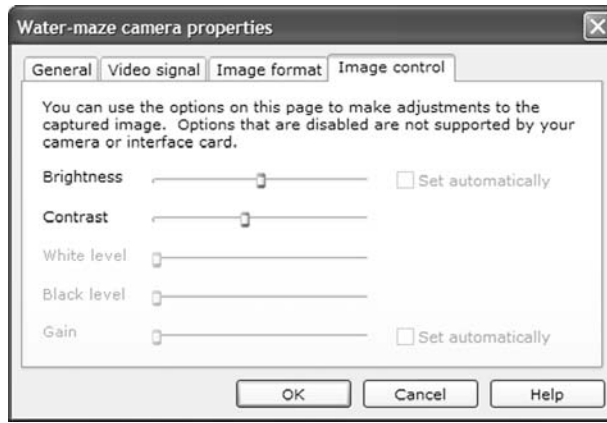


Figure 4.1. The video properties window

Adjustments are made using the *Video properties* window (see figure 4.1), which can be accessed by right clicking on the video picture, and selecting **Properties** from the menu that appears.

Making the video image smaller

The options on the Image format page of the Video properties window – see figure 4.2 – can be used to reduce the size of the image being provided by a camera or digitiser. This can be useful when:

- You are using a slow computer, as a full size image may be too complex for it to process.
- You are using multiple cameras and lots of full sized images simply won't fit on the screen.

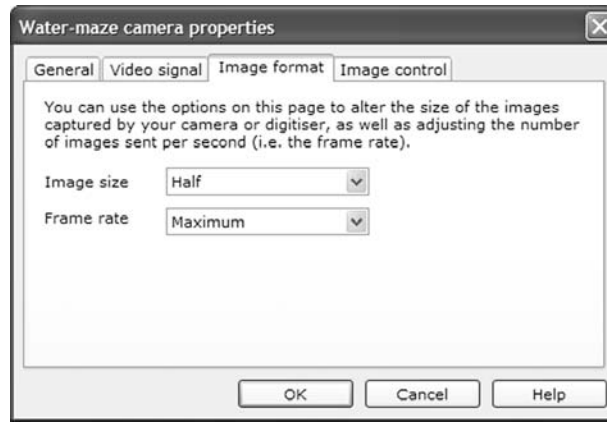







Figure 4.2. The Image format page of the Video properties window.

Flipping the image

In some cases the image shown by your camera may appear to be upside down or back to front. While this doesn't make any difference to the tracking it can be very confusing to look at. You can flip the image both vertically and horizontally using the   buttons shown at the top of the image pane.

Zooming in

If your camera includes a zoom or vari-focal lens then you should use it to zoom in or out as this will give the best image quality. Otherwise, you can use ANY-maze's digital zoom, which is controlled using the   buttons shown at the top of the image pane. When you zoom, some of the area visible to the camera will disappear off the sides of the image and you may need to *pan* the image so you can see the apparatus – you can pan using the  button.

Cropping the image

It's often the case that the apparatus doesn't fill the entire image, as is the situation in figure 4.3, for example.

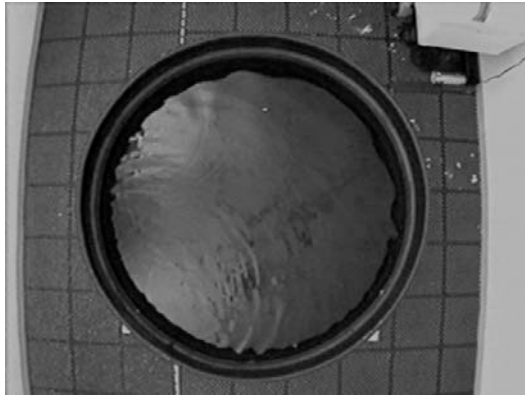


Figure 4.3. In this image the areas to the left and right of the water-maze could be cropped.

In this case it's a good idea to crop the excess areas as this reduces the amount of the data the computer has to process. However, when cropping an image take care that you leave a border around the apparatus – as in figure 4.4.

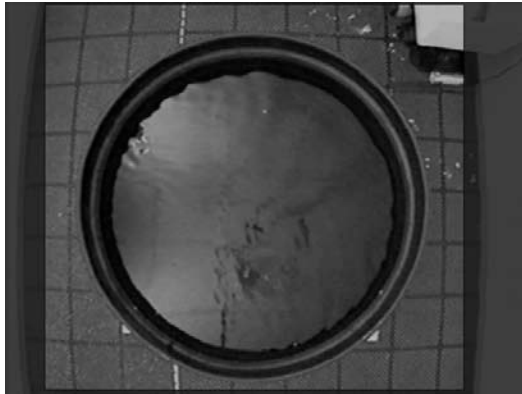



Figure 4.4. The same image as figure 4.3 but with areas that should be cropped shaded.

To crop an image simply use the mouse to select the area you want to keep – this will shade the area that will be cropped, as in figure 4.4. Having cropped an image, all other protocol elements that use it will only show the unshaded area.

Erasing grid lines or cage bars from an image

If a piece of apparatus, such as an open field, has grid lines painted onto its floor, these may interfere with the tracking – although this is generally only the case if the animal's colour lies between the colour of the floor and the grid lines – for example a light grey rat on a white background with black grid lines, as in figure 4.5.

In this case ANY-maze can electronically remove the grid lines. This is also necessary if an animal is being viewed through bars or a wire mesh.

To remove grid lines or cage bars click the  button and follow the instruction given.

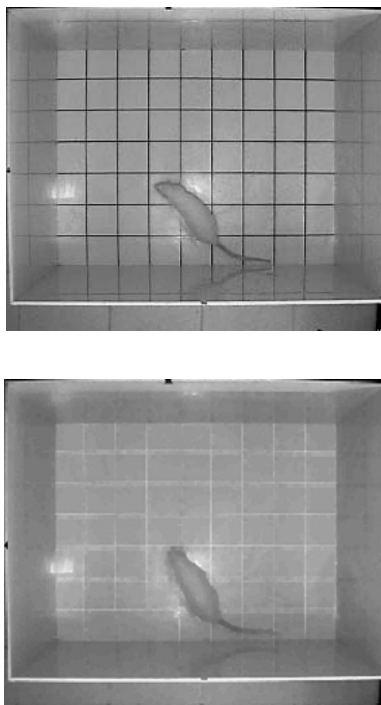


Figure 4.5. In the open field in the top image the dark grid lines will interfere with the tracking. In the bottom image they have been electronically removed.

Working with images that show more than one piece of apparatus

If you plan to work with multiple apparatus you may want to set up a single camera that can see more than one of them. For example, in figure 4.6, a single camera can easily see into the four open fields.

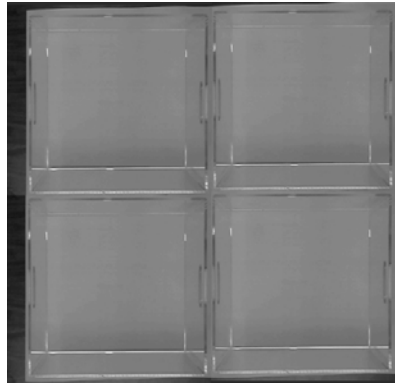


Figure 4.6. A single camera can see into all four of these open fields

However, in ANY-maze a single video source must only show a single piece of apparatus, so in this situation you need to do the following:

1. Add a video source to your protocol, call it Box 1 and select the camera as the Source of video images. Of course this will show the image of all four boxes.

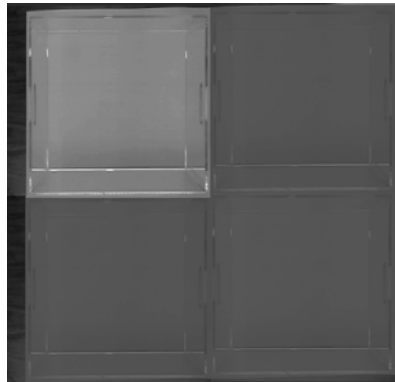


Figure 4.7. The image from figure 4.6 with everything except the top left box cropped.

2. Crop the image (see above) so that just the top left box is visible, as in figure 4.7 (the area that will be cropped from the image is shown shaded).
3. Add a second video source to the protocol, call it Box 2 and select the same camera as its Source of video images.
4. Crop the image so that just the top right box is visible.
5. Repeat this process for Box 3 and 4, in each case cropping the image so just the appropriate box is shown.
6. Now you will have four video sources each of which shows just one of the boxes. You'll then need to add four Apparatus elements to the protocol (one for each box) selecting the appropriate image source for each one.

Joining two images

In some situations a single camera is unable to see into all of a piece of apparatus. For example, it's common to put a place preference box inside a sound proof cabinet, but then a single camera can't be mounted far enough away that it can see both sides.

To overcome this you can create a *montage video source* – this is a video source in which the images from two cameras have been joined together. To do this:

1. Add a video source to the protocol that shows the image from one of the cameras. Adjust the video image and crop the picture as required. Note: It's usually necessary to reduce the image size (see above) otherwise the joined image will simply be too big to fit on the screen.
2. Add a second video source that shows the image from the second camera. Adjust this image and crop the picture as required.
3. Add a *Montage video source* and select the two normal video sources you created in the previous steps to be the constituent video sources – you can choose how they're joined together, side by side or one above the other.

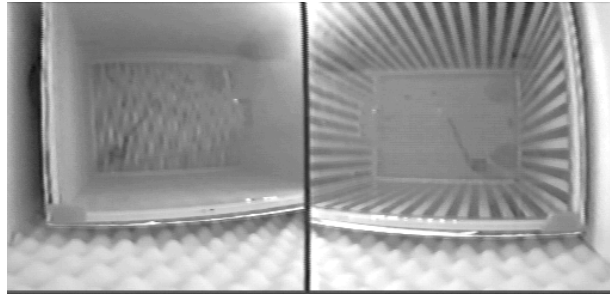


Figure 4.8. In this place preference box two cameras have been used each showing one side of the box. The images have then been joined using a montage video source.

Apparatus

Working with multiple apparatus

ANY-maze can be used with up to 16 pieces of apparatus simultaneously.

To use multiple apparatus you simply need to add an *Apparatus* element to the protocol for each one and then select the video source that shows its image (remember each video source must show the image of just one piece of apparatus).

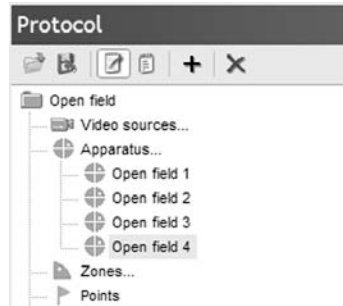


Figure 4.9. Example of a protocol that includes four pieces of apparatus.

Controlling tests using a key and/or an external switch

For each piece of apparatus you can optionally select an 'F' key from the computer's keyboard, and/or an external switch, to control testing.

- ❑ Keys and switches work identically and are usually used to start the test, although their action is in fact dependent on the state of the test – for example while a test is running pressing the key will ask you whether you wish to end the test.
- ❑ ANY-maze supports a range of devices that you can use to connect an external switch to your computer - including the computer's printer port, which offers a simple and cheap solution.
- ❑ External switches can be connected to a long cable allowing you to place them near, or even on, the apparatus so you can press them quickly at the start of a test.

Zones

A zone identifies an area of the apparatus that interests you. ANY-maze can report many different measures for a zone - search the on-line help for `Zone measures` to see a complete list.

Specifying when an animal is in a zone

The simplest way to determine when an animal is in a zone is to use its centre point – the orange dot that tracks the animals during the test. If the dot is inside the zone the animal is considered to be in the zone. Although this method often works well, in some circumstances it can suffer from a problem of *spurious* zone entries.

Imagine a situation in which the animal is straddling a zone boundary such that the dot is just outside the zone. Now the animal moves ever so slightly and the dot enters the zone. It then reverses its slight move and the dot exits the zone. If the animal repeats this pattern, lots of *spurious* zone entries will be counted.

Another issue that arises when using the centre of the animal to score zone entries is correctly identifying where to position the zone borders so entries only occur in the right situation. For example, in the plus maze an arm entry is normally counted when all four feet are in the arm, but with a dot marking the animal's centre how can you score this. Usually people simply move the zone border up the arm to try to compensate but this is rather an arbitrary solution and depends on the animal size, how much it stretches, etc.

In ANY-maze both these issues, spurious entries and precise entry scoring, are addressed in the same way - by allowing you to specify the percentage of the animal that must enter the zone for an entry to be scored, and the amount of the animal that must *remain in the zone* to *prevent* an exit from being scored.

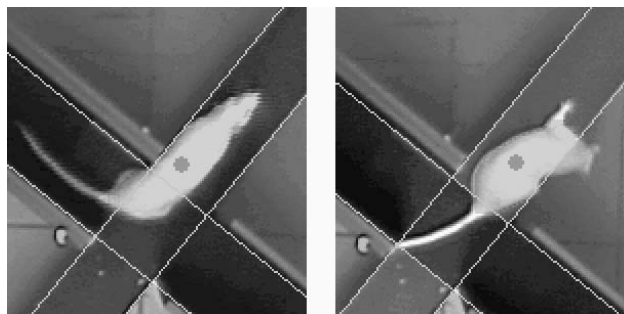


Figure 4.10. In the left-hand image the centre of the animal, indicated by the dot, has entered the open arm but ANY-maze doesn't count this as an entry because less than the required amount of the animal, 85% in this case, is in the zone. In the right-hand image ANY-maze does consider the animal to be in the zone, which it indicates by shading the zone.

For example, in the plus maze you might define the percentage of the animal that must enter the arm for an entry to be scored as 85% (which generally approximates well to the 4-paws-in-the-arm rule) and the percentage that must remain in the arm for an exit not to be scored as 70%. It's probably clear how the 85% value will solve the issue of precise zone entries (see figure 4.10), but how does this prevent the spurious entries problem? The key to this is the difference between the two values used. What we're saying is that 85% of the animal must enter the arm for an entry to occur but provided 70% remains there no exit will be scored. Thus, after entering the arm the animal must make quite a significant exit movement before an exit will occur.

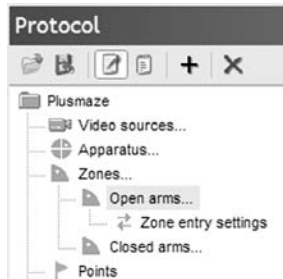


Figure 4.11. Zone entry settings are specified in a sub element automatically included in each zone.

You actually specify the method used to score zone entries and exits using the Zone's *Zone entry settings* sub element in the protocol – see figure 4.11.

Hidden zones

In some apparatus you may have areas in which the camera will be unable to see the animal. For example, a tunnel connecting the two sides of a place preference box, a nest box, the dark side of a light dark box, etc.

Clearly if the camera can't see the animal then it won't be able to track it, but you may want to know such things as how often the animal entered the hidden area and how long it spent there. You can achieve this by defining a *Hidden zone*.

Each protocol can only include one hidden zone and ANY-maze will automatically consider the animal is in it if it can't find it anywhere else in the apparatus.

To specify a hidden zone, simply check the **This is a hidden zone** button in the Zone settings.

Unlike normal zones, for which you can define zone entry and exit percentages (see the previous section), hidden zones only allow you to specify a zone exit percentage. This is because the entry is automatically scored when ANY-maze can't find the animal. In this case, the exit

percentage allows you to control how much of the animal must leave the hidden zone before it's deemed to have made an exit. This setting can help to prevent spurious exits from being scored if the animal just peeks out of the zone.

Zones that change position between tests

In most situations a zone is located in a fixed position within the apparatus. For example a *Centre zone* in an open field will always be located in the same place. However, in some apparatus zones can move about. This is often the case in apparatus that includes a reward zone, and can also be the case in a water maze, where the position of the island can change.

Normally, when you define a zone in ANY-maze, you simply indicate the zone location in the apparatus by clicking on it in the apparatus map, but if the zone can adopt different positions then this clearly isn't going to work.

This issue is addressed by specifying that a zone is *moveable*. You can then add *Position* sub-elements to the zone to define each of the different position that it can adopt – see figure 4.12.

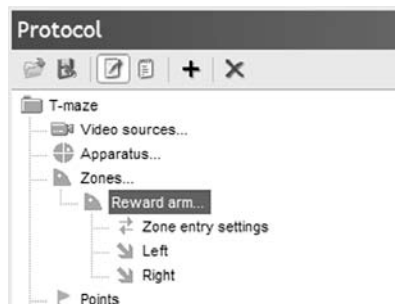


Figure 4.12. In this T-maze the reward arm can adopt one of two positions: left or right.

To actually define a zone as moveable you should select one of the options in the drop down list shown on the zone settings page – see figure 4.13.

Settings

Zone name : Reward arm

If this is a hidden zone, a tunnel for example, then check the box below. Whenever it can't see the animal, the system will assume it's in this zone.

☐ This is a hidden zone

In some protocols a zone may not always be located in the same position (a platform in a water-maze, for example). In these cases you should tell the system how the position varies by selecting an appropriate option from the list below, and then use the **+** button above to add one 'Zone position' for each position that the zone can adopt.

Position varies between (but not within) the animals

Position of the zone remains the same in all tests

Position varies between (but not within) the animals

Position varies within (and possibly between) the animals

Position follows the position of other moveable zones

the appropriate areas in each of the apparatus maps shown on the right.

If you want help setting up this zone then select the button in the main toolbar or click this link.

Figure 4.13. The options that can be specified when defining a moveable zone.

A full description of the different types of moveable zones is beyond the scope of this manual (they're documented in the on-line help), but essentially the differences relate to how you will define the zones position in different tests. For example, specifying that the zone can move about between but not within animals, means you will be able to define the zone's position for each animal just once, no matter how many trials the animals actually receive.

Ending a test when the animal enters a zone

In goal orientated testing, such as the water-maze, you will often want to end a test when the animal enters a specific location. To do this you should first ensure that the location has been defined as a zone. You will then need to define an *Event* to detect when the animal enters the zone, and an *Action* to end the test when it does so – see *Events and Actions* later in this chapter for more details.

How zones work with multiple apparatus

When working with multiple apparatus it's important to understand that you don't need to create separate zones for each one, rather you create a single zone and specify where in each piece of apparatus it's located – see figure 4.14.

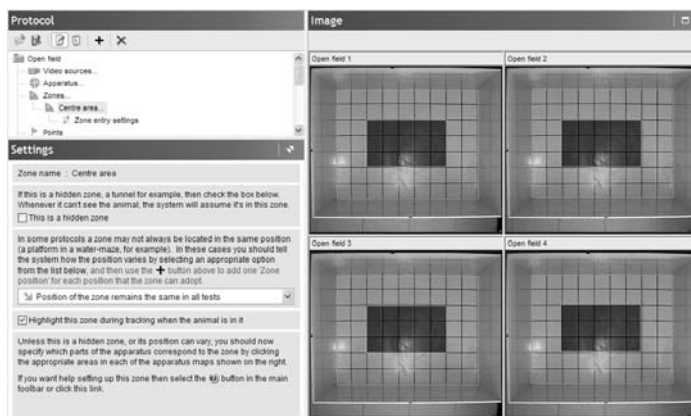


Figure 4.14. Defining the position of the Centre zone in a protocol that includes four open fields.

Points

Points simply allow you to define a specific position in the apparatus that interests you. ANY-maze will then report a range of measures for the point.

How points work with multiple apparatus

Like for zones, if your protocol includes multiple apparatus, you still only need to define each point once – you will then be able to identify where it's located in each piece of apparatus individually.

Sequences

As the name implies, sequences are used to detect a sequence of movements within the apparatus. For example, a sequence could be used to detect rotations around a water maze.

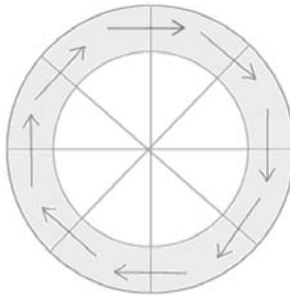


Figure 4.15. The steps in a sequence to detect rotations around a water-maze. In this case the sequence would be defined as “Can start at any step”, and “Can be performed in either direction”.

Defining the steps in a sequence

A sequence consists of a number of *Steps* where each one defines an area within the apparatus that the animal must enter in order to perform the step. A sequence is completed when all the steps have been performed.

A step's area can be defined either by selecting any arbitrary areas in the apparatus map or by specifying that the area is the same as that of a zone.

Usually defining a step by selecting areas of the apparatus map is easiest, but using a zone can be very useful if the location of the step can change between tests as you can use a moveable zone to define it.

For example, if you have a reward arm in a T maze, you may be interested to know whether the animal goes directly from the stem of the T into the reward arm, a simple sequence could tell you this. But the reward arm may sometimes be on one side of the T and sometimes on the other – this wouldn't be a problem as you could define the Reward arm as a moveable zone and then specify the sequence steps as *Stem of the T* to *Reward arm zone*.

Keys

Keys are designed to allow you to score behaviours that ANY-maze can't detect automatically. For example, you could use a key to score when the animal is grooming.

Using keys

The idea behind a key is very simple - you define a key on the computer's keyboard to represent a behaviour and then, during the test, you press the key while the behaviour is being exhibited. For example, you might choose to use the G key for grooming, so, while the animal is grooming you'd simply hold the key down.

In fact, you don't have to hold down a key during the behaviour, if you prefer you can specify that the key *toggles*, so the first time you press it you are indicating that the behaviour has started, and the second time that the behaviour has ended. This can be particularly useful if the behaviour could last for a long period of time.

How ANY-maze scores key behaviours

ANY-maze will report various measures for a key behaviour, for example how many times it occurred and how long it occurred for. These measures are available not just for the apparatus as a whole but also for any zones you have defined. So, for example, you could see how much time the animal spent grooming in the centre of an open field as well as how much time it spent grooming overall.

Using keys with multiple apparatus

If your protocol includes multiple apparatus then, for each key behaviour you define, you will need to define one key on the keyboard for each apparatus. For example, G for *Grooming in Open field 1*, L for *Grooming in Open field 2*, etc. – see figure 4.16.

Settings

Key name : Grooming

Key stroke for Open field 1 : G

Key stroke for Open field 2 : L

Key stroke for Open field 3 : W

Key stroke for Open field 4 : P

Specify how you'd like this key to work:

Simple - key activity is occurring while key is pressed

☐ Require the Ctrl key to be pressed in order to register this key

☒ This behaviour should be counted as activity

If you want help setting up this key then select the button on the main toolbar or click this link.

Figure 4.16. When defining a key for a protocol that includes multiple apparatus, you need to specify a different key stroke for each one.

Switches

There are two types of switches in ANY-maze, *Input switches* and *Output switches*.

Input switches are used to detect when external switches or sensors are activated. For example, you could install some photo-beams in your apparatus and use an input switch to detect when they're broken by a rearing animal.

Output switches are used to control external devices; for example, an output switch could turn on a buzzer, a shocker or a lamp.

Connecting switches

To physically connect a switch to your apparatus you need something called a *Digital I/O device*. ANY-maze supports a number of these devices, the best of which is the Switch and Sense 8/8 (available direct from Stoelting). This device connects to a USB port on your computer and allows you to connect up to 8 input switches or sensors and up to 8 outputs. For example, if you have 4 boxes containing a lever, a lamp and a shock grid then you could connect them all to just a single Switch and Sense 8/8 device. The levers would connect as inputs and the lamps and shockers as outputs. ANY-maze would then be able to sense when a lever is pressed and would be able to turn the lamps and shockers on and off.

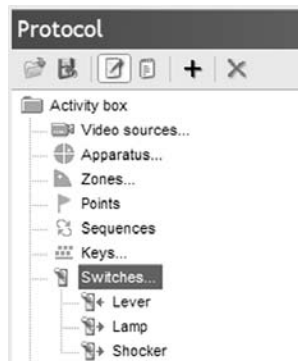


Figure 4.17. A protocol that includes an input switch element to detect presses of a lever, and two output switch elements to control a lamp and shocker, respectively.

Settings

Output switch name : Shocker

Output for Box 1 : Switch & sense 8 device 0 - Relay 0

Output for Box 2 : Switch & sense 8 device 0 - Relay 1

Output for Box 3 : Switch & sense 8 device 0 - Relay 2

Output for Box 4 : Switch & sense 8 device 0 - Relay 3

Default state for this output : ☒ Off ☐ On

When activated what should this switch do:

☐ Turn the output on

☐ Turn the output off

☒ Pulse the output on for the following duration (ms) : 500

☐ Pulse the output off for the following duration (ms) :

If you need help setting up this output switch then select the button on the main toolbar or click this link.

Figure 4.18. The settings for the shocker switch. Note that there are 4 pieces of apparatus, boxes 1 – 4, each of which has an individual shocker.

Specifying how to react to inputs and when to switch outputs

The Switches protocol elements are only used to specify how devices *connect* to your computer, they aren't used to specify what ANY-maze should actually do when, for example, a photo-beam is broken. Nor do they specify when, for example, the program should turn a shocker on and off. These things are controlled by *Events and Actions*, which are described later in this chapter.

Test settings and options

The test settings and options define what ANY-maze tracks, what it scores and what information it displays and records.

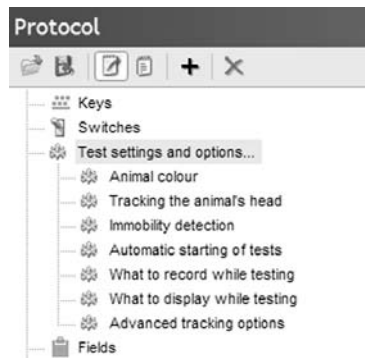


Figure 4.19. The Test settings and options

Animal colour

The animal colour actually defines whether the animal is lighter or darker than the apparatus background. This is usually easy enough to specify, but it may be that the animal is not uniform, for example, it has light and dark patches of fur, or that the background is not uniform, perhaps because the light levels vary substantially as in figure 4.20. In this case, you can choose an option for **The background and/or the animals are not uniform**.

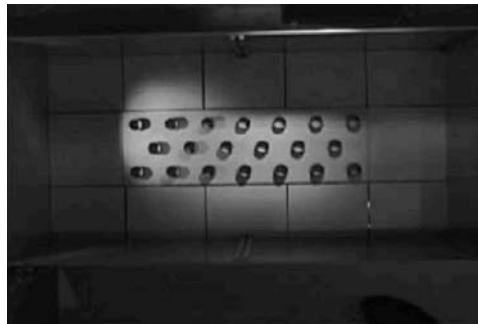


Figure 4.20. In this apparatus the uneven lighting makes it impossible to specify whether the animals are lighter or darker than the background. Therefore the “Background and/or the animals are not uniform” option must be used.

Another situation that can arise is that the animals are themselves different - some are lighter than the background and others darker. For example, you may be performing an experiment with different strains. In this case you can choose either to have ANY-maze automatically detect the animal colour at the start of the test, or you can specify the animal colour manually by entering it into the *Animal treatments and data table*, which is accessible from the *Experiment management home page*.

Tracking the animal's head

Ordinarily ANY-maze will track the animal's centre of gravity, however, in some cases you may wish to track the position of the animal's head as well. This is possible, although there are some limitations:

- It only works with rodents.
- There must be at least moderate contrast between the animal and the background of the apparatus.

Immobility detection

ANY-maze can detect periods when an animal is immobile and report various measures both for the apparatus as a whole and also for the individual zones.

Defining exactly when an animal is immobile is less obvious than it might at first appear and ANY-maze uses two values to do this. The first value is the percentage of the animal that must remain in the same place for it to be considered immobile and the second value is how long this situation must last before a period of immobility should be deemed to have begun.

For example, if you are interested in detecting when the animal stops moving about within the apparatus (i.e. no locomotor activity) then you would probably use around 60% for the first value and maybe 2 seconds for the second one. Thus if at least 60% of the animal remains in the same place for at least two seconds, it would be considered immobile.

To detect 100% immobility the image quality must be good and there must be a high level of contrast between the animal and the background.

On the other hand, if you wished to know when the animal freezes then you would probably set the first value to 100% and the second to a short period, such as ¼ second.

Note that, to detect 100% immobility the image quality must be good and there must be a high level of contrast between the animal and the background.

Automatically starting tests

ANY-maze can automatically start a test when the experimenter leaves the camera's view. This is very useful as it means you don't need to rush back to the computer and press a key to get the test to begin.

However, there may be circumstances where automatic test start is simply not possible (perhaps you can't leave the camera's view) in which case you can choose to start tests manually.

There are three ways to do this: clicking the **Start test** button; pressing an 'F' key on the computer's keyboard, or pressing an external switch connected to some type of *Digital I/O device* (or to the computer's parallel port). The latter option has the advantage that you don't need to return to the computer as the switch can be on a long cable.

Choosing what to record while testing

While a test is running ANY-maze will try to detect the animal in every image it receives from the camera – this will typically be 30 images per second. However, it's often not necessary to record all these positions as they'll probably be very close together. By default ANY-maze will actually record at most 10 positions per second, but you can change this to as few as 1 position per second or as many as 30, if you wish.

As well as recording the position of the animal, you can optionally choose to record a digital video of a test as well. This can be particularly useful in long-term tests where you don't actually watch the test while it's occurring.

Recording videos of tests can use a lot of space on your hard disk. You should ensure there's plenty of free space (gigabytes) before starting your experiment.

Choosing what to display while a test is running

During a test ANY-maze can display:

- The position of the animal's centre of gravity.
- The position of the animal's head.
- The area of the video image it considers to be the animal.
- An indicator if the animal is immobile.
- An indicator when the animal is in a zone.
- An indicator when a behaviour key is active.
- An indicator when an input switch is active.

You can choose which, if any, of the above you would like shown.

Advanced tracking options

The last of the *Test settings and options* is the *Advanced tracking options*. This is used to set some more obscure options whose usage is beyond the scope of this manual – refer to the on-line help for details.

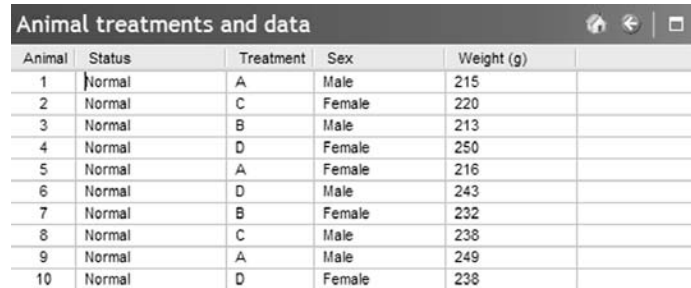
Fields

In most experiments you will wish to record additional information about your animals, such as their weights, and possibly about the tests, such as the temperature of the water in a water maze.

In ANY-maze you can add *Fields* to your protocol for these items. Doing so will cause the program to include places for you to record the data in the experiment.

For example, if you wish to record the weights of your animals you would add a field for it to your protocol. This field would probably be called Weight, would be defined as being recorded for the animals (rather than for tests), would be numeric and would have units of grams.

Having added a Weight field, then, in the experiment, ANY-maze would include a location for you to enter the weight of each animal – see figure 4.21.



Animal	Status	Treatment	Sex	Weight (g)
1	Normal	A	Male	215
2	Normal	C	Female	220
3	Normal	B	Male	213
4	Normal	D	Female	250
5	Normal	A	Female	216
6	Normal	D	Male	243
7	Normal	B	Female	232
8	Normal	C	Male	238
9	Normal	A	Male	249
10	Normal	D	Female	238

Figure 4.21. The protocol used in this experiment includes fields for the animals' sex and weights. Therefore ANY-maze includes columns in the Animals Treatments and data page where these values can be entered.

Using field data in analysis

If you include a *Numeric field*, such as weight, in your protocol, then you will be able to analyse it in the results. For example, you will be able to check whether there was any significant difference in the weights of the animals in different groups.

Another type of field is a *Choice field*. In this case the field's definition includes a number of possible values for the field. For example, *Sex* would be a choice field with two possible values *Male* and *Female*. When you include a choice field you will be able to use it as an independent variable in the results, so for example, you could determine whether there was a difference between the Male and Female animals in they time spent in a certain zone.

Stages

In ANY-maze all experiments consist of a number of Stages and all stages consist of a number of repeated trials. In simple terms, each animal is tested through all the trials in each stage. Of course, you can choose to define a protocol that includes just a single stage that consists of just one trial, in which case each animal will be tested just once (indeed this is the default setting).

Repeated trials

If you do wish to test your animals repeatedly then you will need to set up multiple stages and/or multiple trials in each stage.

Whether you should use multiple trials in a single stage or multiple stages or a mix of the two depends on a number of factors.

- The trials in a stage must all be of the same length (although you can define situations that will end a trial prematurely, such as the animal finding the island in a water maze).
- You may wish to divide your experiment into stages simply because it makes sense to do so. For example, if you test all your animals in the water-maze for 5 minutes, every week, for four weeks then you could either create four stages or one stage with four repeated trials. But if in the first three trials the animals were being trained and in the last trial you are testing their memory retention then you might choose to define two stages, *Training* and *Retention*, with the former having 3 trials and the latter just 1. This wouldn't be strictly *necessary*, but it would make more sense for you.

How animal proceed through the trials in a stage

If the animals are to be tested more than once in a stage then there's a question of what order they should be tested in. For example, you might choose to test animal 1 repeatedly until it has completed all of its trials before you proceed to animal 2, or you might choose to test all the

animals in their first trial before proceeding to the second one. ANY-maze accommodates both these options as well as a combination option in which the animals proceed through their trials in groups.

Ending the trials in a stage automatically

In some experiments you may wish to give the animals repeated trials in a stage until they achieve some goal. So, some animals might have 5 trials in the stage, others 6 or 7, etc. (although you will probably still have an absolute maximum number of trials).

ANY-maze not only allows you to set up stages with such variable numbers of trials but it can also detect when the trials for an animal should end. For example, you might define the goal as finding the island in a water maze within 20 seconds in two consecutive trials. If you defined this as a *Stage end rule* then ANY-maze would automatically detect when an animal achieves the goal and stop testing it. Such *Stage end rules* are defined using the *Stage end rules* sub-element that's automatically included in all stages – see figure 4.22.

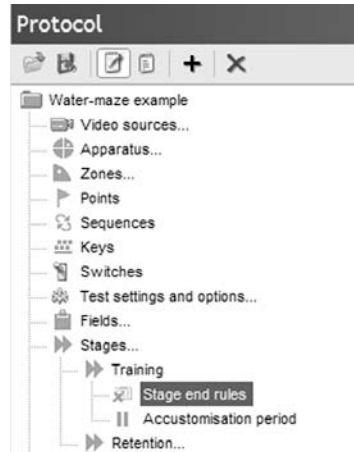


Figure 4.22. All stages automatically include a *Stage end rules* sub-element.

Events and actions

Events and actions are two different protocol elements that are closely linked. An *Event* is used to define something that you want the software to **detect** and an *Action* is used to define something you want it to **do** when an *Event* occurs.

Ending a water maze test when the animal finds the island

The easiest way to understand events and actions is through an example. In the water maze it's normal to end the test when the animal finds the island and this is a classic situation where events and actions are required.

In this case you would define an event to detect entry into the island zone (you would, of course, need to have defined the island as a zone), and you would then define an Action that would end the test when the *Finds island* event occurs.

Defining events

An event is said to be *triggered* by some type of occurrence during a test. There are over a hundred different triggers available covering a wide range of possibilities, such as the animal being immobile for a certain period, a behaviour key being pressed a certain number of times, an input switch being activated, etc. – see figure 4.23.

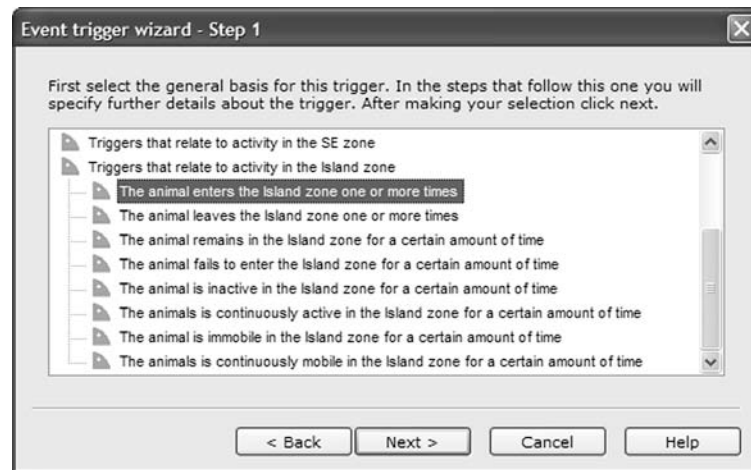


Figure 4.23. Some of the possible triggers for an event.

Taking actions

Actions are triggered by events. In fact, a single action can be set to be triggered by more than one event. So, for example, you could specify that the test should end if the animal either “*Enters zone A*” **or** “*Fails to enter zone B for 60 seconds*”. Alternatively you could require that two, or more, events must occur before the action will be triggered.

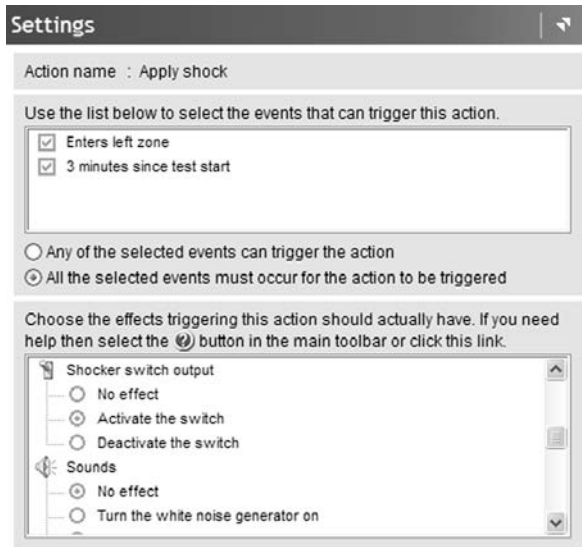


Figure 4.24. In this protocol the animal will be shocked if it enters the left zone three minutes, or more, after the test starts.

There are a number of actions that can be taken when an *Action* is triggered, including:

- Ending, pausing or un-pausing the test.
- Starting, stopping, pausing, or un-pausing the video recorder.
- Adding a label to a video recording (so you quickly jump to the event in the recording when you review it).
- Activating or deactivating an output switch. (You will recall that these can control devices such as buzzers, lamps, shockers, etc.).
- Playing a sound.

Calculations

Although ANY-maze will automatically calculate a large range of different measures, there may be occasions when you want to calculate a result it doesn't supply as standard.

For example, in a plus maze protocol you would probably define an "Open arms zone" and this would cause ANY-maze to report, amongst other things, the "Time in the open arms". However, in the plus maze it's quite common to report the "Percentage of time in the open arms" rather than the absolute time, but this is a value which ANY-maze *doesn't* include as standard.

The answer is to define a calculation to derive the measure. In this case it would be:

```
( Time in the open arms / Test duration ) * 100
```

Or, if you prefer to exclude the time in the centre of the maze, then:

```
( Time in the open arms / ( Test duration - Time in  
centre ) ) * 100
```

Any measure can be included in a calculation, as can the results of other calculations and also the values of numeric fields.

Using functions

As well as using the standard mathematical operators, add, subtract, multiply and divide, ANY-maze also includes a number of functions that can be used in calculations. In the main these generate a result based on the value of a measure in more than one test. For example, you can use the *Mean* function to calculate the average result for a measure across a series of trials.

Another useful function is *ResultForPeriod* which will return the result of a measure for just a specific period of a test, perhaps for the period from 20 seconds to 90 seconds

If you want to analyse tests across time you may find that the "Segment of test" feature of the Analysis element is more useful than the ResultForPeriod function.

Analysis

The analysis element is used to set various options that relate to how ANY-maze analyses test results.

Analysing tests across time

Normally ANY-maze reports a single result for each measure for each test. So, for example, it will report the total time in the open arms in a plus maze test. However, you might be interested to know how this measure varies across the course of the test – for example, does the animal spend more time in the open arm towards the start of the test, or more at the end, or is there no difference.

To answer this type of question you can divide tests into equal length segments and then analyse results for each one. So, in a 5-minute plus maze test you might divide the test into 30-second segments and then compare the results between each of them – see figure 4.25.

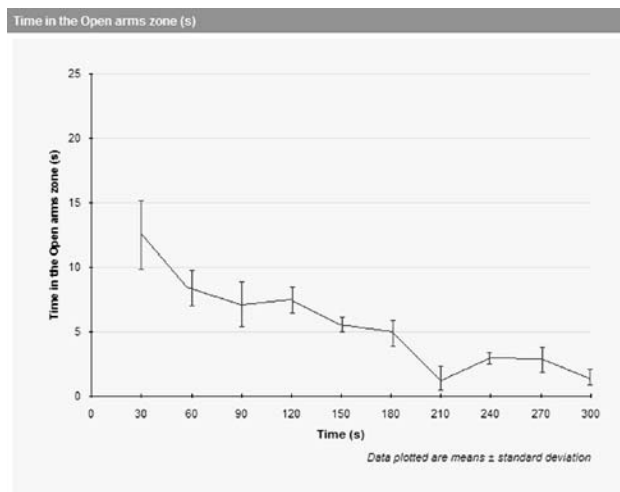


Figure 4.25. Time in the open arms of a plus maze analysed across the duration of the test. For this analysis the test has been divided into 30-second segments.

To actually perform this type of analysis you simply need to choose *Segment of test* as an independent variable when analysing results, however, the *segment length* that will be used is defined in the *Analysis element* of the protocol.

Other analysis options

The other analysis options are beyond the scope of this manual, although you will of course find detailed information about them in the on-line help.

Results reports and data

Choosing the information included in standard reports

The standard experiment management reports, such as the *Test schedule report* and the *Test details report*, include certain information by default. However, in many cases you will want to change this so that information pertinent to your protocol is shown. You can do this using the relevant sub-elements of the *Results reports and data* element in the protocol. For example, in a plus maze you might wish to add the result of the “*Percentage of time in the open arms*” calculation to the results shown on the *Test details report*.

Choosing the rows and columns shown on the Data page

By default the Data page includes one row for every test in the experiment and shows a small sub set of the available measures in its columns.

Most often you will want to alter the columns shown to include those measures that interest you. Although you can do this directly on the Data page, you can also define the columns as part of the protocol, which has

the advantage that any experiments you create based on the protocol will already have the appropriate measures selected.

Defining results reports in the protocol

Normally you will create results reports in ANY-maze in an ad hoc way. For example, you will simply go to the *Results page* and choose a report type, select the measure or measures that interest you and then view the resulting report.

However, when designing your protocol you may already know of certain measures that you will almost always want to view. For example, in a plus maze you'll probably always look at the *Percentage of time in the open arms*, the *Number of entries into the open arms*, and so on. That being the case you could define a report within the protocol that will show these results. This would have the advantage that any experiment you perform using the protocol will already have this report defined and you'll simply need to click a link in the Results page to view it – see figure 4.26.

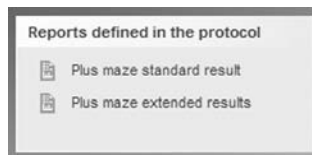


Figure 4.26. Any results reports that are defined in the protocol appear in a panel on the main Results page - a single click will create the relevant report.

Defining report sets

If you perform tests manually you're probably in the habit of gathering up all the results, reports, notes etc. and filing them together when the experiment is complete. This way if you want to refer back to the experiment everything's in one place. Of course, with time you'll perform lots of experiments and, at the end of a year you may have a couple of

files labelled *Water-maze experiments*, or whatever. ANY-maze lets you do a similar thing electronically using *Report sets*.

A report set defines a group of ANY-maze reports that you want to create for an experiment. These can include such things as the *Protocol report*, which defines the protocol you used, the *Test schedule report*, which details the tests performed, the *Animal and Test details reports*, which contain full information about the animals and tests in the experiment, and any *Results reports* defined in the protocol. Having specified which reports you want to include in a report set you can *publish* it – which will cause ANY-maze to create all the reports and save them in HTML format, (like a web-site) with links to each one in an index page. Indeed it will even maintain a master index of all the experiments you’ve published, again in HTML format.

Thus with time, your master index will hold a list of all your experiments and from it you’ll be able to quickly navigate to any experiment and within the experiments to any report. And because all this is stored in HTML format you can view these reports using a web browser - so there’s no need to use ANY-maze.

Indeed by choosing to publish report sets to a server computer in your lab, you could make your results available to your students, colleagues or collaborators. They’d just need a web browser to view them.

Summary

If you’ve read this entire chapter you’ll now have a good idea about what facilities are available within ANY-maze and you’ll have a clear understanding of just how important the protocol is to the system.

Although reading through all the features that a protocol *can* include makes the system seem extremely complex, it’s worth remembering that no single protocol is likely to use more than a relatively small sub set of them. Also, having designed a protocol you can save it and then reuse it

again and again, so time spent getting the protocol right is rarely time wasted.

Your Distributor is:
Braintree Scientific, Inc.
PO Box 850498, Braintree, MA 02185
781-917-9526
Email: Info@braintreesci.com
Web: www.braintreesci.com

End note

Doubtless, after reading this manual you will still have many unanswered questions: *Can I edit a protocol I've saved, Can I delete an animal from an experiment, How do I save data to SPSS...*

You'll find all the answers in the ANY-maze on-line help. There are over 800 pages of information covering every aspect of the program. Alternatively you can just contact us and we'll quickly provide you with an answer.

Making ANY-maze better

We're committed to making ANY-maze as good as possible and we'd really appreciate hearing your comments about this manual – and, of course, about the software too. If you have any criticisms, suggestions or ideas please contact us at techsupport@anymaze.com