

**Description of the
sample experiment**

Morris water maze test

for EthoVision XT 16

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For EthoVision XT 16

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Get started

MORRIS WATER MAZE TEST XT160

The installation USB stick contains the backup file (*.evz) of the EthoVision XT sample experiment **Morris water maze test XT160**. This experiment is an example of how you can automate a memory test with a water maze and EthoVision XT.



Key features of this experiment

- **Video files.** The backup experiment contains 20 video files. For this you need about 250 MB space on disk.
- **Experimental groups.** The experiment contrasts two groups of two rats each, Young (5-7 months old) vs. Aged (c. 24 months old). The aim of the experiment was to quantify the ability of young animals to learn the location of the platform relative to the older animals.

- *Training trials.* In the original experiment, each rat was trained in four days (Day 1 to 4) and four times per day (t1 to t4). In this highlights experiment only d1 and d4, t1 and t4 are shown.
- *Probe trials.* Each rat was tested on Day 5, for two minutes.

What you can learn with this experiment

- Define zones in the water maze that are important for analysis, for example the quadrants, or the Whishaw's corridors.
- Define the platform of the water maze to control the end of data acquisition.
- Plan your trials in advance, and assign experimental groups and other variable values to each subject.
- Define behavioral endpoints typical of this test, including swim distance, time in quadrants, thigmotaxis behavior and search errors: cumulative errors in training trials, average errors in probe trials.
- Use the Nest function in the Data profile to pin-point specific parts of the swim paths, for example the first few seconds of each track.
- Use the dependent variable *Heading to point* in the Analysis profile to quantify heading angle errors.
- Make charts and heatmaps for presenting the key results of this study.

OPEN THE SAMPLE EXPERIMENT

The sample experiment is normally present on your computer after you have installed EthoVision XT.

Procedure

To open the experiment the first time you must choose the Restore Backup function:

1. In EthoVision XT, choose **File > Restore Backup**. Browse to the folder:
C:\Users\Public\Documents\Noldus\EthoVision XT\Experiments\Sample Experiments
2. Select the file **Morris water maze test XT160.evz** and click **Open**.
3. Choose where to save the experiment.

The default location is C:\Users\Public\Documents\Noldus\EthoVision XT\Experiments.

Notes

- If you do not find the **evz** file, on the EthoVision XT installation USB stick browse to the **Sample Experiments** folder and copy **Morris water maze XT16o.evz** to your computer. Then in EthoVision XT, choose **File > Restore Backup**. Select the **evz** file and click **Open**.
- For more information about using EthoVision XT in a water maze test, in Windows under **Noldus** choose **EthoVision XT 16 Other Documentation** and open the **Application Manual - EthoVision XT 16**.

OTHER SAMPLE EXPERIMENTS FOR ETHOVISION XT

You can download other sample experiments on the website of Noldus IT. These sample experiments are examples of commonly used tests with EthoVision XT.

1. Browse to my.noldus.com. Login or register first.
2. Under **Downloads** choose **Sample projects** and in the new screen click **EthoVision XT**.
3. Download the **evz** file you are interested in.
4. Follow the procedure above to open the experiment with the **Restore Backup** function.

NOTE Not all sample experiments may immediately be available. Check regularly our web site for more sample experiments.

ACKNOWLEDGMENTS

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www.tnrg.hu

Explore the sample experiment

EXPERIMENT SETTINGS

Choose **Setup > Experiment Settings**.

Under **Tracked Features**, **Center point detection** is selected. That is typical for water maze experiment where it is not essential to track the nose and the tail base of the subjects.

Because the video contains the image of one water maze, we have selected **1** under **Arenas**. Because we track one subject at a time, we have selected **1** under **Subjects**.

ARENA SETTINGS

Choose **Setup > Arena Settings > Open Arena Settings 1**.

The outline of the arena has been defined with a circle.

The Arena Settings contains multiple layers of zones, named Zone groups. With zone groups you can define zones that overlap but are considered as separate units, for example a quadrant and a corridor that crosses it. Within a zone group, zones are mutually-exclusive.

Zone groups

- **Platform layer** which includes the platform zone.
- **Quadrants layer** with the four zones (quadrants).
- **Thigmotaxis layer**, which includes a zone named Border zone, which you can use to quantify the time spent or the distance covered next to the pool walls.
- Three more zone groups **Whishaw's corridor N**, **Whishaw's corridor E** and **Whishaw's corridor S**. They contain one zone each, a rectangle that connects the release point (N, E or S respectively) with the platform zone. Although they contain one zone each, they must be separate groups because the corridors slightly overlap.

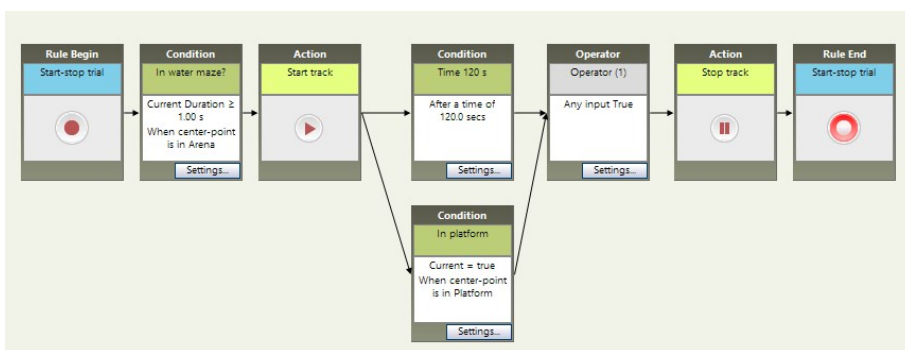
TIP To create an experiment with predefined zones, use the function **File > New from template > Use a predefined template**. Next, choose **Water maze** with **Platform, quadrants, corridors, border**.

TRIAL CONTROL SETTINGS

Choose **Setup** > **Trial Control** > open one of the two in the list.

Trial Control for Training trials

This Trial Control Settings profile was used to acquire the 16 training trials. In a training trial, tracking starts 1 second after the animal has been detected in the pool, and stops when the animal is found on the platform. If the animal does not find the platform, the trials is stopped automatically after 120 s. This condition is expressed in a branched sequence in the figure below.



Trial Control for Probe trials

Tracking starts when the subject is detected for one second in the water pool. Tracking ends after 120 s. With this Trial Control profile you make sure that probe trials have a fixed duration.

DETECTION SETTINGS

Choose **Setup** > **Detection Settings** > **Open Detection Settings 1**.

Detection Settings 1

The detection method **Differencing** was used. This allows better detection of a Lister-hooded rat and can cope with light gradients within the arena better than other methods.

TRIAL LIST

Choose **Setup > Trial List**.

In the Trial List you can plan your trials in advance. Currently the Trial List includes the twenty trials acquired. To add more trials, click the **Add Trials** button.

Trial List								
Add Trials...		Add Variable		Add Videos...		Import External Data...		
							Show	
Label	System		User-defined	User-defined	User-defined	User-defined	System	
	Video file		Group	Day	Trial type	Release point	Arena settings	
Description	The name and path of the video used for acquisition		Experimental group: The subject is either Aged (c. 24 months old) or Young (5-7 months old)	1 to 4 (only 1 and 4 included here)	Training (t) or probe (p). During training, each animal is tested in 4 trials per day; t1 to t4 (only t1 and t4 are shown here).	Sides of the water pool: S (at the bottom of video), N, E, W.	The arena settings used for acquisition	
Type			Text	Text	Text	Text		
Format								
Predefined Values			Aged; Young	Day 1; Day 2; D	t1; t4; p; t2; t3	N; S; E; W		
Scope	Trial	Subject	Subject	Subject	Subject	Subject	Trial	
Trial	Subject	No.						
Trial 1	Subject 1	1	...ter maze test XT140/Media Files/0115_LH22_d1_t1.mp4	Aged	Day 1	t1	S	Arena Settings 1
Trial 2	Subject 1	2	...ter maze test XT140/Media Files/0115_LH22_d1_t4.mp4	Aged	Day 1	t4	E	Arena Settings 1
Trial 3	Subject 1	3	...ter maze test XT140/Media Files/0115_LH40_d1_t1.mp4	Young	Day 1	t1	S	Arena Settings 1
Trial 4	Subject 1	4	...ter maze test XT140/Media Files/0115_LH40_d1_t4.mp4	Young	Day 1	t4	E	Arena Settings 1
Trial 5	Subject 1	5	...ter maze test XT140/Media Files/0115_LH34_d1_t1.mp4	Aged	Day 1	t1	S	Arena Settings 1
Trial 6	Subject 1	6	...ter maze test XT140/Media Files/0115_LH34_d1_t4.mp4	Aged	Day 1	t4	E	Arena Settings 1
Trial 7	Subject 1	7	...ter maze test XT140/Media Files/0115_LH46_d1_t1.mp4	Young	Day 1	t1	S	Arena Settings 1
Trial 8	Subject 1	8	...ter maze test XT140/Media Files/0115_LH46_d1_t4.mp4	Young	Day 1	t4	E	Arena Settings 1
Trial 9	Subject 1	9	...ter maze test XT140/Media Files/0118_LH22_d4_t1.mp4	Aged	Day 4	t1	E	Arena Settings 1
Trial 10	Subject 1	10	...ter maze test XT140/Media Files/0118_LH22_d4_t4.mp4	Aged	Day 4	t4	N	Arena Settings 1
Trial 11	Subject 1	11	...ter maze test XT140/Media Files/0118_LH40_d4_t1.mp4	Young	Day 4	t1	E	Arena Settings 1
Trial 12	Subject 1	12	...ter maze test XT140/Media Files/0118_LH40_d4_t4.mp4	Young	Day 4	t4	N	Arena Settings 1
Trial 13	Subject 1	13	...ter maze test XT140/Media Files/0118_LH34_d4_t1.mp4	Aged	Day 4	t1	E	Arena Settings 1
Trial 14	Subject 1	14	...ter maze test XT140/Media Files/0118_LH34_d4_t4.mp4	Aged	Day 4	t4	N	Arena Settings 1
Trial 15	Subject 1	15	...ter maze test XT140/Media Files/0118_LH46_d4_t1.mp4	Young	Day 4	t1	E	Arena Settings 1
Trial 16	Subject 1	16	...ter maze test XT140/Media Files/0118_LH46_d4_t4.mp4	Young	Day 4	t4	N	Arena Settings 1
Trial 17	Subject 1	17	...ter maze test XT140/Media Files/0119_LH22_probe.mp4	Aged	Day 5	p	S	Arena Settings 1
Trial 18	Subject 1	18	...ter maze test XT140/Media Files/0119_LH40_probe.mp4	Young	Day 5	p	S	Arena Settings 1
Trial 19	Subject 1	19	...ter maze test XT140/Media Files/0119_LH34_probe.mp4	Aged	Day 5	p	S	Arena Settings 1
Trial 20	Subject 1	20	...ter maze test XT140/Media Files/0119_LH46_probe.mp4	Young	Day 5	p	S	Arena Settings 1

Variables

The Trial List is also useful to add variables important for analysis, for example the experimental group, or the training day, or the treatment etc. To add a variable, click the **Add Variable** button and enter the appropriate value in each cell of the table.

The following variables are already defined:

- **Group.** Specifies whether the trial belongs to a Young or Aged group.
- **Day.** Specifies on which day the trial was performed (**Day 1 to Day 5**). Training trials were done on Days 1 to 4; of these, only **Day 1** and **Day 4** are included in this sample experiment. All probe trials were performed on Day 5.
- **Trial type.** Specifies the type of trial, either training (**t**) or probe (**p**). For reach training day, four trials were performed. These are marked with **t1 to t4**; only **t1** and **t4** are included in this sample experiment.
- **Release point.** Specifies where the subject was released.

You can use one or more of those variables to filter data and create analysis groups.

Acquire the data

ACQUIRE ONE TRIAL

Procedure

1. Choose **Acquisition > Open Acquisition**.
2. In the Acquisition Settings window, under **Settings**, click the video button and choose one of the video files. Choose between a training trial and a probe trial. Video files marked with t1 or t4 are of training trials.
3. Click the **Add Trial** button.



4. Under **Settings**, choose which Trial Control Settings you want to use, depending on whether the video files represents a training trial or a probe trial.
5. Click the **Start trial** button.



The trial ends automatically based on the Trial Control Settings.

Notes

- The video files are stored in C:\Users\Public\Documents\Noldus\EthoVision XT\Experiments\Morris water maze test XT16o\Media Files.
- You can check the newly-acquired trials in the Trial List (Trial 21, 22, etc.).
- **OPTIONAL** For each new trial, enter the values of Group, Training trial, etc. This allows you to analyze the new trials more efficiently together with the others.

ACQUIRE A SERIES OF TRIALS (BATCH MODE)

You can also acquire the tracks from a group of videos using the Trial List.

1. In the Trial List, click the **Add Videos** button and select the videos. In the Trial List, specify which Arena Settings, Detection Settings and Trial Control Settings you want to use (take note of which video is a training trial vs. probe trial).
2. Then **Choose Acquisition > Open Acquisition** and choose **Track all planned trials**, then click the **Start trial** button.

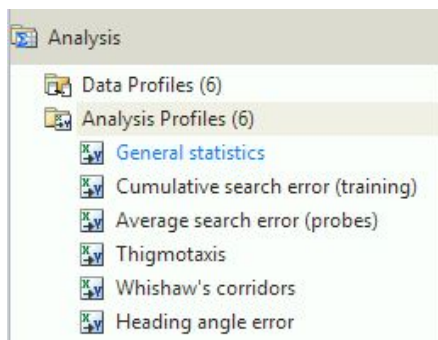


3. All videos are processed according to the sequence in the Trial List.

Analyze the data

ANALYSIS PROFILES

Each section below refers to the variables defined in the corresponding Analysis profile under **Analysis**. Often you combine such variables with a Data profile to select a specific segment of your tracks.



How to obtain analysis results

1. Choose the analysis you want to carry out (for example, choose **Analysis > Results > Statistics and Charts**).
2. Select the Data profile and the Analysis profile from the lists on the toolbar (see the next sections).

For more information about analysis, in the EthoVision XT Help (Choose **Help > Help topics**) choose one of the items in the table of contents:

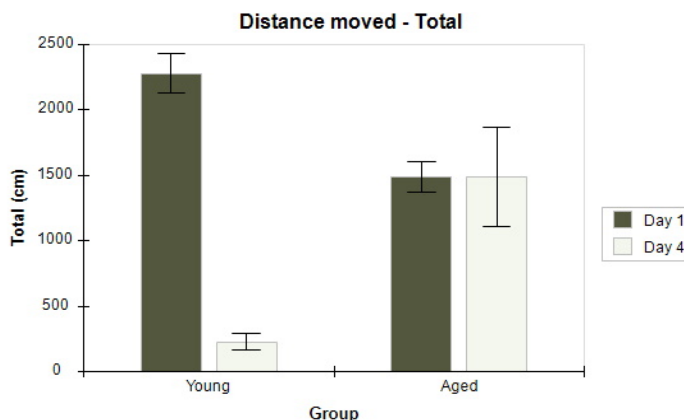
- For analysis functions, choose **Introduction to Data Analysis**.
- For quantitative analysis: see **Calculate Statistics**.
- For heatmaps and track plots: **Visualize Data**.
- For analyzing intervals within tracks: **Analyze Track Segments**.

The different Analysis profiles with their predefined analysis variables are described below.

GENERAL STATISTICS

Choose **General statistics** to view statistics of distance moved (swim path length), velocity and the time spent in quadrants. Combine this analysis profile with the Data profile **Default**

Data profile to view statistics per trial. Use other Data profiles to view statistics per group or for training/probe trials.



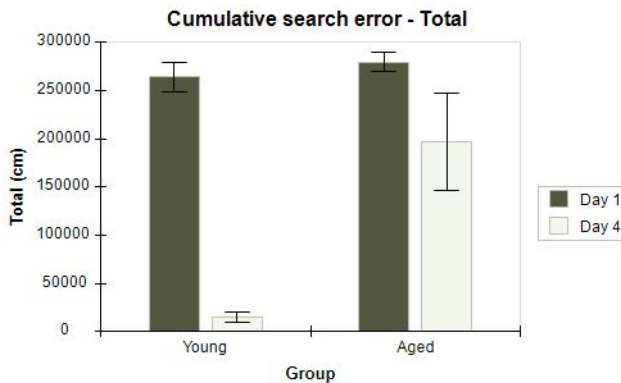
- The variable named **Time in quadrants** is of type *In zone*. In such a variable you can set a zone exit threshold. Use this option to remove false re-entries resulting from random movements of the body point around the boundaries between quadrants. For details, see the EthoVision XT Help.
- The variable named **Velocity bins with JavaScript state** is a of type *JavaScript State*. The JavaScript code processes the data of *Velocity* and groups the results in four bins based on some thresholds, which you can change. You can use this variable to create a frequency distribution of velocity data and compare that between experimental groups. To edit the variable, double-click the name and edit the thresholds at the top of the window. Combine this Analysis profile with the data profile **Training trials - d1 vs d4**.

CUMULATIVE SEARCH ERROR

With **Cumulative search error** you calculate the sum of the distance of the subject from the platform at each sample time. This is an index of proximity to the platform in training trials and is comparable to the Cumulative search error defined by Gallagher *et al.* 1993 (*Behavioral Neuroscience* **107**: 618-626).

Combine this Analysis profile with the Data profile **Training trials - d1 vs d4**.

Choose **Analysis > Results > Statistics & Charts** and click **Group Statistics & Charts**. There you see that the search error in young rats strongly decreased from day 1 to day 4 of the training days, while in aged rats the error is still large at day 4.

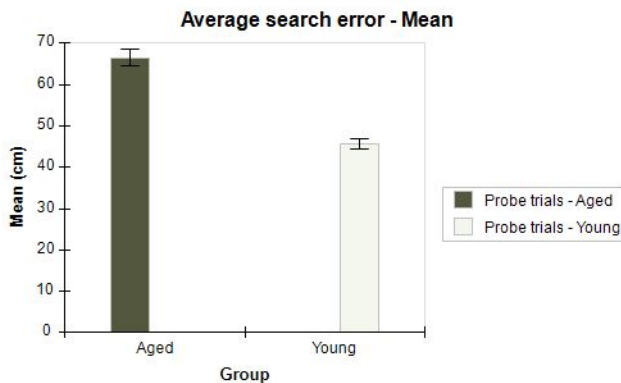


AVERAGE SEARCH ERROR

With **Average search error** you calculate the average of the distance of the subject from the point where the platform is supposed to be in probe trials. That is the same as the average search error for probe trials defined by Gallagher *et al.* 1993 (*Behavioral Neuroscience* **107**: 618-626).

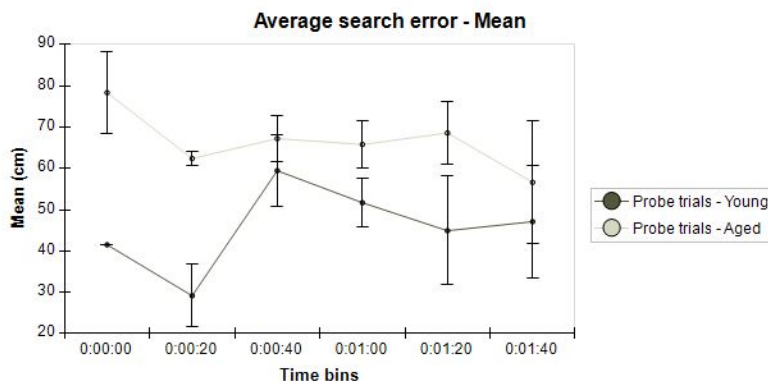
Combine this Analysis profile with the Data profile **Probe trials** or **Probe trials with Time bins**.

Choose **Analysis > Results > Statistics & Charts** and click **Group Statistics & Charts**. There you see a clear difference in the search error between aged and young rats.



If you choose **Probe trials with Time bins** from the toolbar, then the average search error is calculated for each 20-s interval from the start of the trial. You can see that the difference

between the groups is largest in the beginning of the trials: young rats start searching immediately in the right spot.



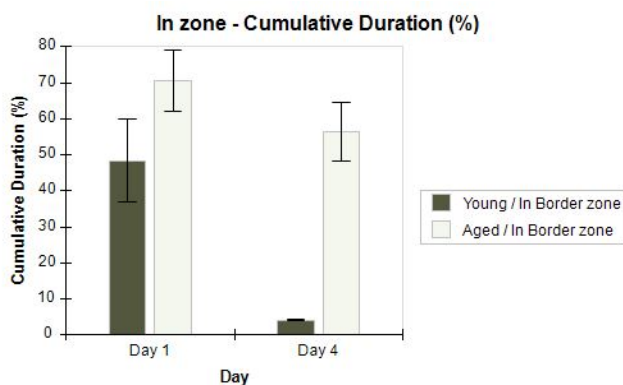
NOTE The values on the horizontal axis represent the starting times of the Time bins. For example, the first pair of points on the left in the chart represent the search error from the start of the track (0:00:00) to 20 s. The second pair of points represent the search errors between 20 s and 40 s, etc.

THIGMOTAXIS

Use **Thigmotaxis** to measure the distance swum and the time spent by the subject next to the walls of the water pool (that is, in the zone named Border zone). This indicates thigmotaxis, a behaviour often interpreted as the absence of a strategy to find the platform because the animals look for wall contact where they presumably feel safer.

Combine this Analysis profile with the Data profile **Thigmotaxis**. Note that this Data profile is set to **Results per zone**, and provides results only for the Border zone, not other zones.

A clear difference appears between the two groups at day 4 of the training trials when considering the time in the border, expressed as percentage of the total time. Note that older rats still show high proportions of thigmotaxis at Day 4.

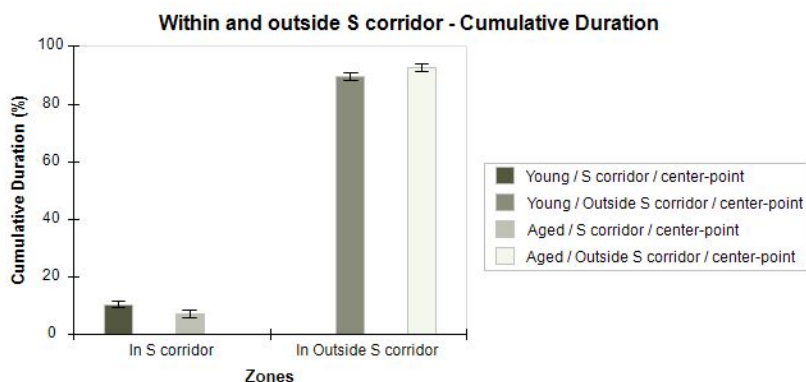


WHISHAW'S CORRIDORS

This Analysis profile makes use of the zones Whishaw's corridors defined in the Arena Settings. In this example the corridor S is used. The aim of this analysis is to measure the time and swim path length in one of the corridors, using the tracks started from the corresponding release point.

Combine **Whishaw's corridors** with the Data profile **Rat released from side S**. This profile filters all tracks that started from the release point S (South). It is also set to **Results per zone** because it must filter the data points collected in the corridor zone **S Corridor** and the complementary zone **Outside S corridor** (that is, the rest of the water pool).

When released from the side S of the water maze, young rats spend more time in the optimal corridor than older rats.



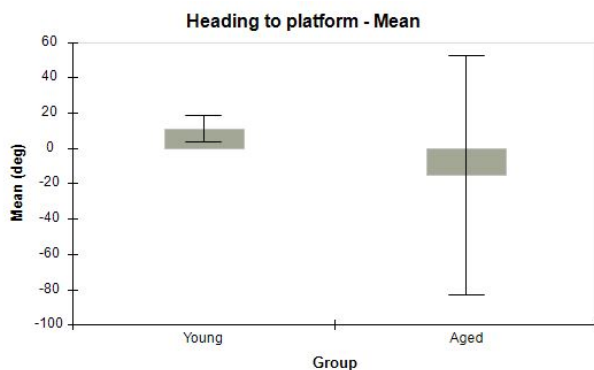
You can create similar Analysis profiles and Data profiles using the corridors N and E and filter the tracks starting from the release points N and E, respectively.

HEADING ANGLE ERROR

The Analysis profile **Heading angle error** includes two variables of type *Heading*: Heading to platform and Heading to center of platform quadrant (that is, quadrant North-West). In both cases they measure the tendency of the subject to move towards a target, rather than the time spent in those zones.

Combine this analysis profile with the Data profile named **Probe trials, first 5 s**. This filters the first 5 seconds of all tracks that belong to the probe trials.

The heading of young rats is close to zero, with small between-trials variation, meaning that young rats point to the target zones immediately after being released in the pool. Older animals show a less clear pattern.



Visualize the data

INTEGRATED VISUALIZATION

To visualize one trial at a time, and play back the track with the video, choose **Analysis > Results > Plot Integrated Data**.

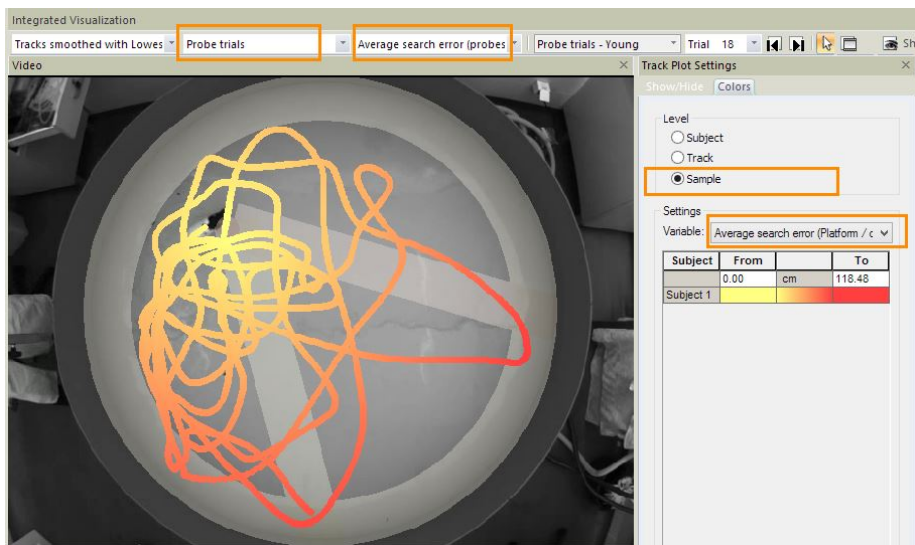
TIP Click the **Jump to end** button to view the entire tracks.



Visualize a variable in the track plot

From the lists on the toolbar, choose one of the Data profiles and one of the Analysis profiles. For example, Data profile Probe trials and Analysis profile Average search error (probes). On the **Colors** tab, under **Level** choose **Sample** and choose **Average search error**.

This way you can display the amount of search error in different colors.

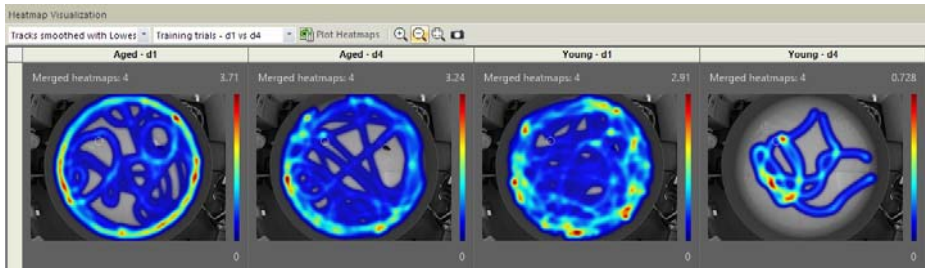


HEATMAP VISUALIZATION

Choose Analysis > Results > Plot Heatmaps.

Training trials: effect s of age and training day

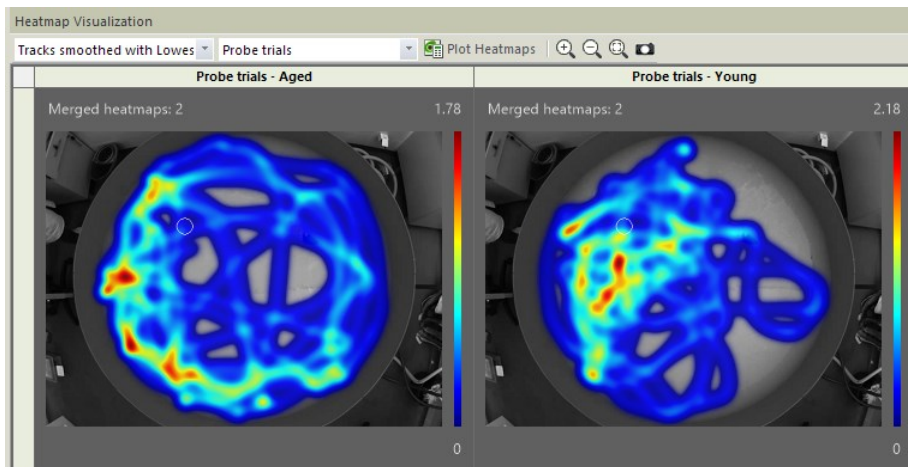
From the list on the toolbar choose **Training trials - d1 vs d4**.



Probe trials: effect of age

A striking pattern also emerges for probe trials.

From the list on the toolbar choose **Probe trials**. There you see a clear hotspot for the group Young, near the location of the target platform.



The scale at the right side of each heatmap helps you quantify the time that the subject spent in a region of the water maze.