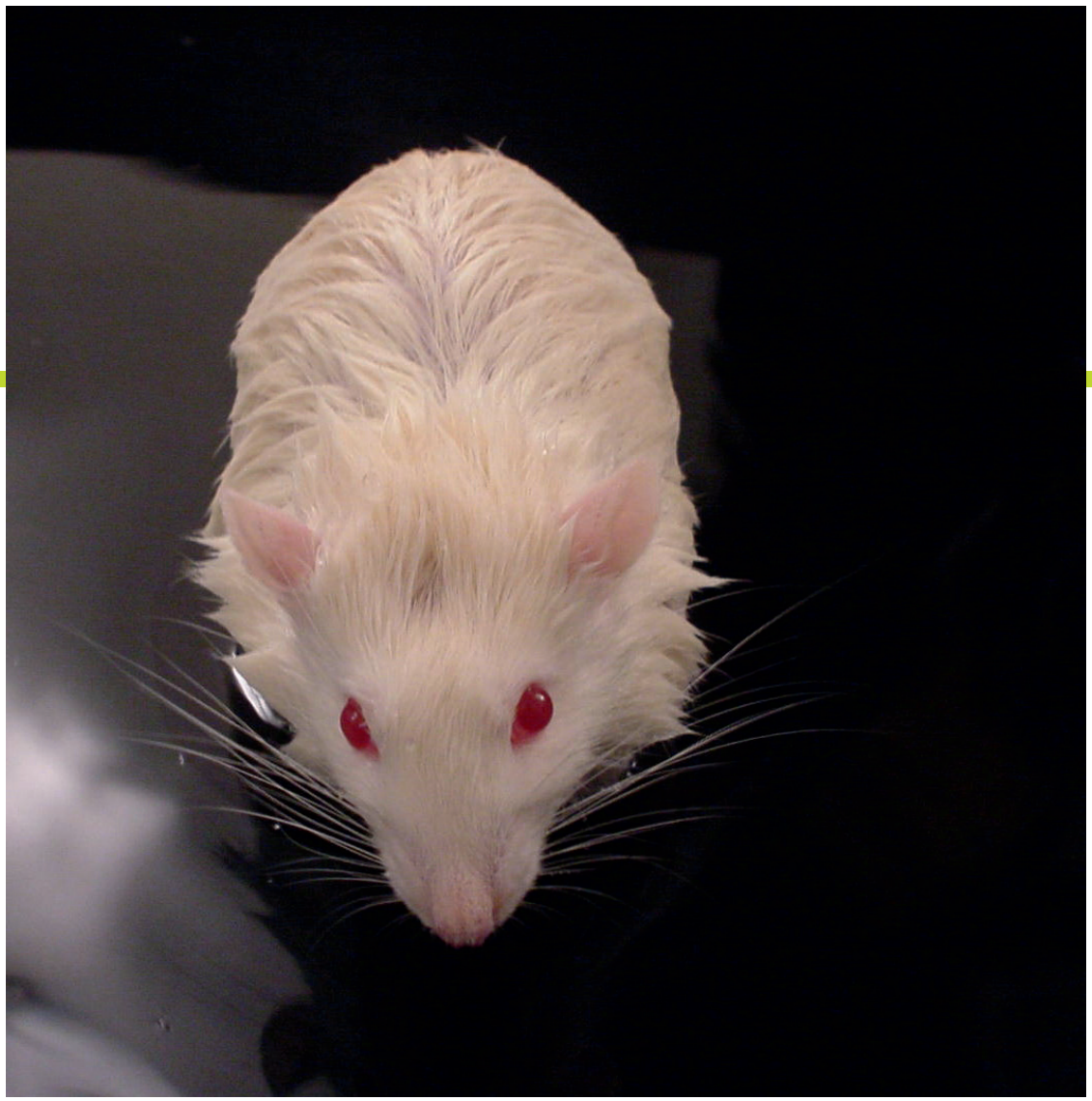


How the water maze is used in Alzheimer's research

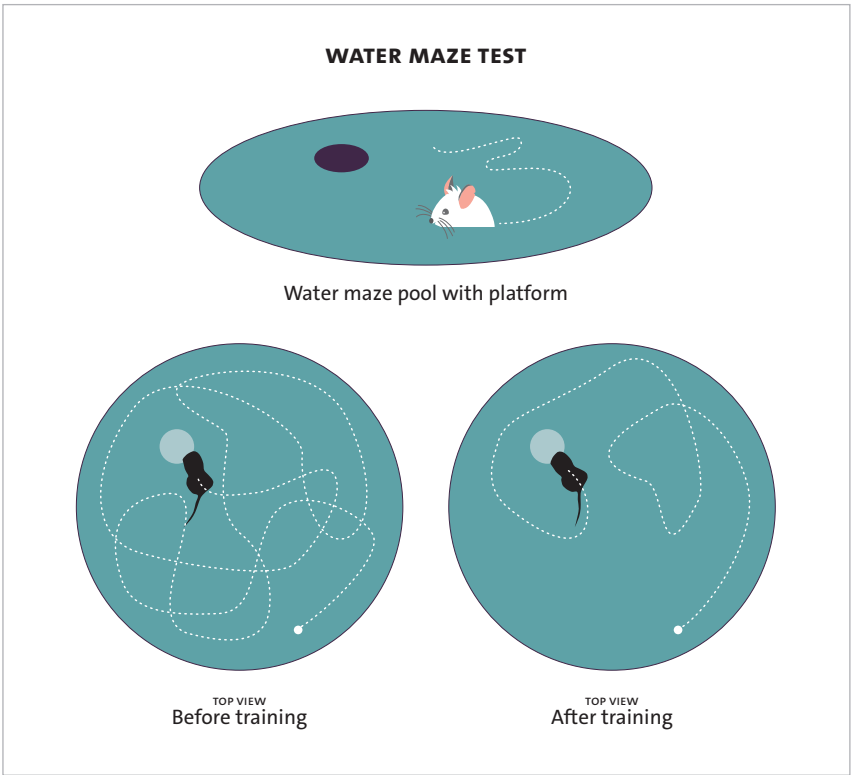
How you can enhance your research with EthoVision XT

A white paper by Noldus Information Technology

UNDERSTANDING THE MORRIS WATER MAZE

Many of the neuro-behavioral studies done on AD involve the water maze test.

First introduced by *Richard Morris* in 1981, the Morris water maze (MWM) remains one of the most widely used behavioral tests for studying spatial memory and learning behavior. During a test, a rat or mouse learns to locate a platform hidden just beneath the water surface of a large pool. Healthy animals learn to locate the platform at an increasingly faster rate over multiple trials. However, there are many factors that can inhibit the ability of the animal to find the platform.



One of these factors is Alzheimer's disease (AD). AD is the most common form on dementia, and one of the most common neurodegenerative diseases. Since AD comes with memory impairments, many researchers use the MWM to study the effects of potential treatments.

HOW TO DO TESTING IN A WATER MAZE

The water maze test typically involves several days of learning trials, followed by a probe trial.

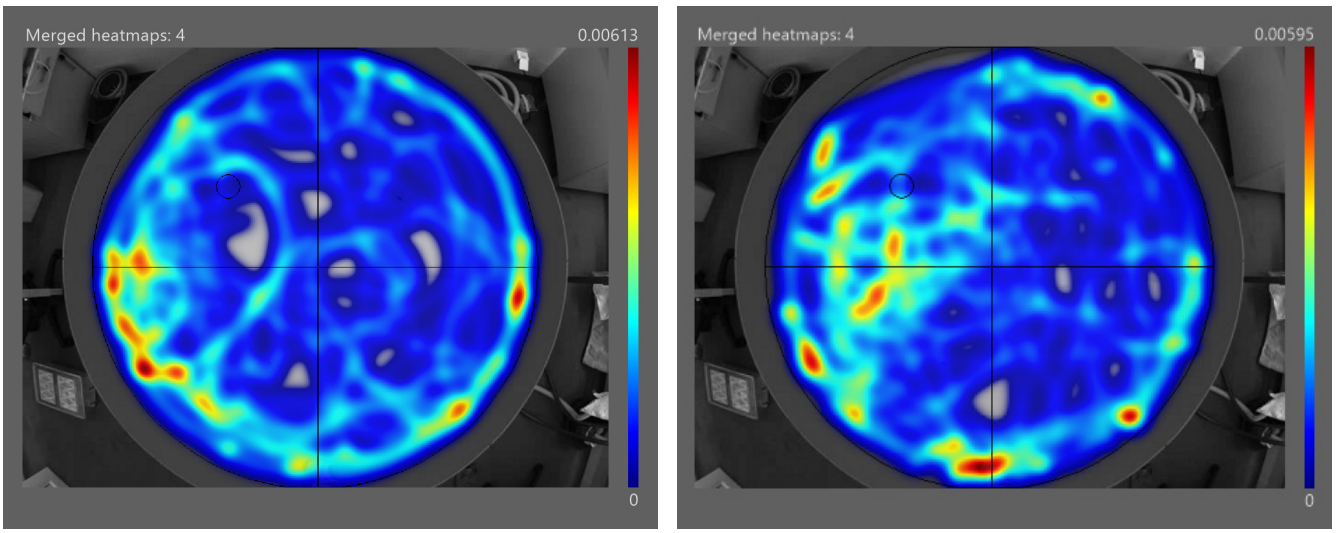
LEARNING PHASE TRIALS

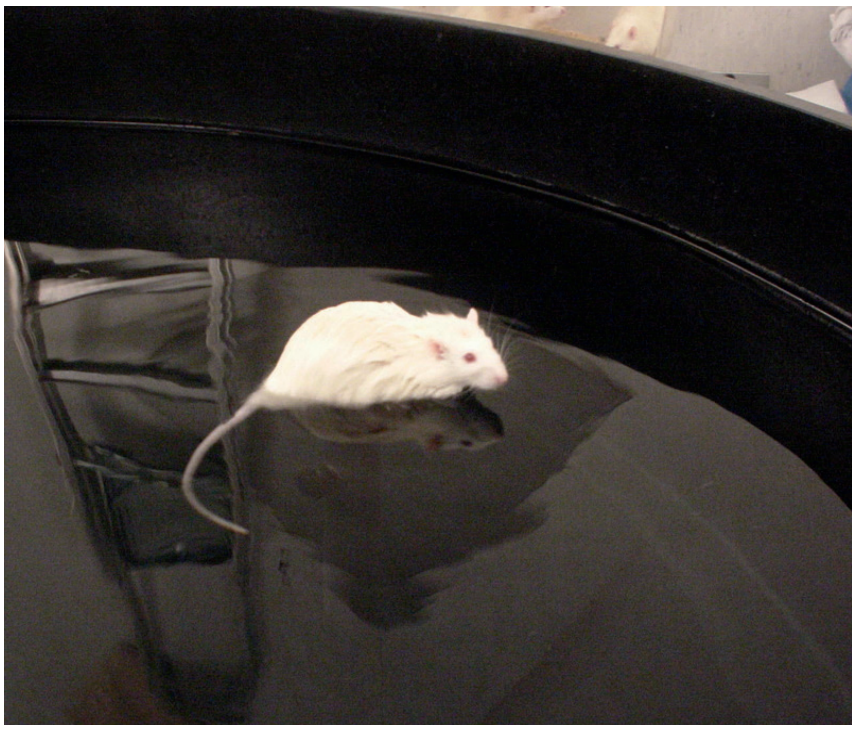
During a reference memory learning phase, the position of the platform doesn't change, although the starting position of the animal might [2]. The animal is usually put into the pool facing the wall. Over the course of four or five days, animals undergo about four trials per day during which the aim is to learn to location of the hidden platform with the help of visual cues [1,2,5]. There is a cut-off time of one [1-4] or sometimes two [5] minutes. If the subject hasn't found the platform by then, the experimenter guides it. Regardless, the animal is left on the platform for 10 to 15 seconds.

PRE-TRAINING

Some researchers use pre-training. For example, Majkutewicz *et al.* [3] used a 60-second trial with a visible platform. Orr *et al.* [4] did pre-training in a rectangular channel with a platform.

Merged heatmaps of swimming patterns in a Morris water maze.





PROBE TRIALS

After 4 or 5 days of training, a probe trial (without a platform) is used to investigate reference memory. Most researcher prefer to have at least 24 hours between the last learning trial and the probe test. Otherwise, it is difficult to differentiate between short- and long-term memory formation.

REVERSAL LEARNING AND WORKING MEMORY

In addition to spatial learning tasks, it is increasingly common to perform addition learning trials. For example, the location of the platform is changed, and the animal is subjected to another set of trials to test reversal learning.

Majkutewicz *et al.* [3] tested working memory learning by changing the position of the platform each day (4 trials per day).

ADVANCED TRACKING IS MORE ACCURATE

PRECISE MEASUREMENT OF ESCAPE LATENCY

The time it takes for an animal to locate the hidden platform, or escape latency, is the most critical parameter in the MWM. While it might seem simple to measure this manually using a stopwatch, this method introduces subjectivity and variability.

Automated video tracking softwares, like EthoVision® XT, automate this process.

> EthoVision XT integrates seamlessly with your experimental setup.

HOW ETHOVISION XT WORKS

EthoVision XT integrates seamlessly with your experimental setup. A camera mounted above the water maze records the animal's movement, either live or from pre-recorded video. The software uses advanced tracking algorithms to:

- Detect the animal's position in real time
- Measure swim speed, path length, and time spend in certain zones
- Monitor activity with ease

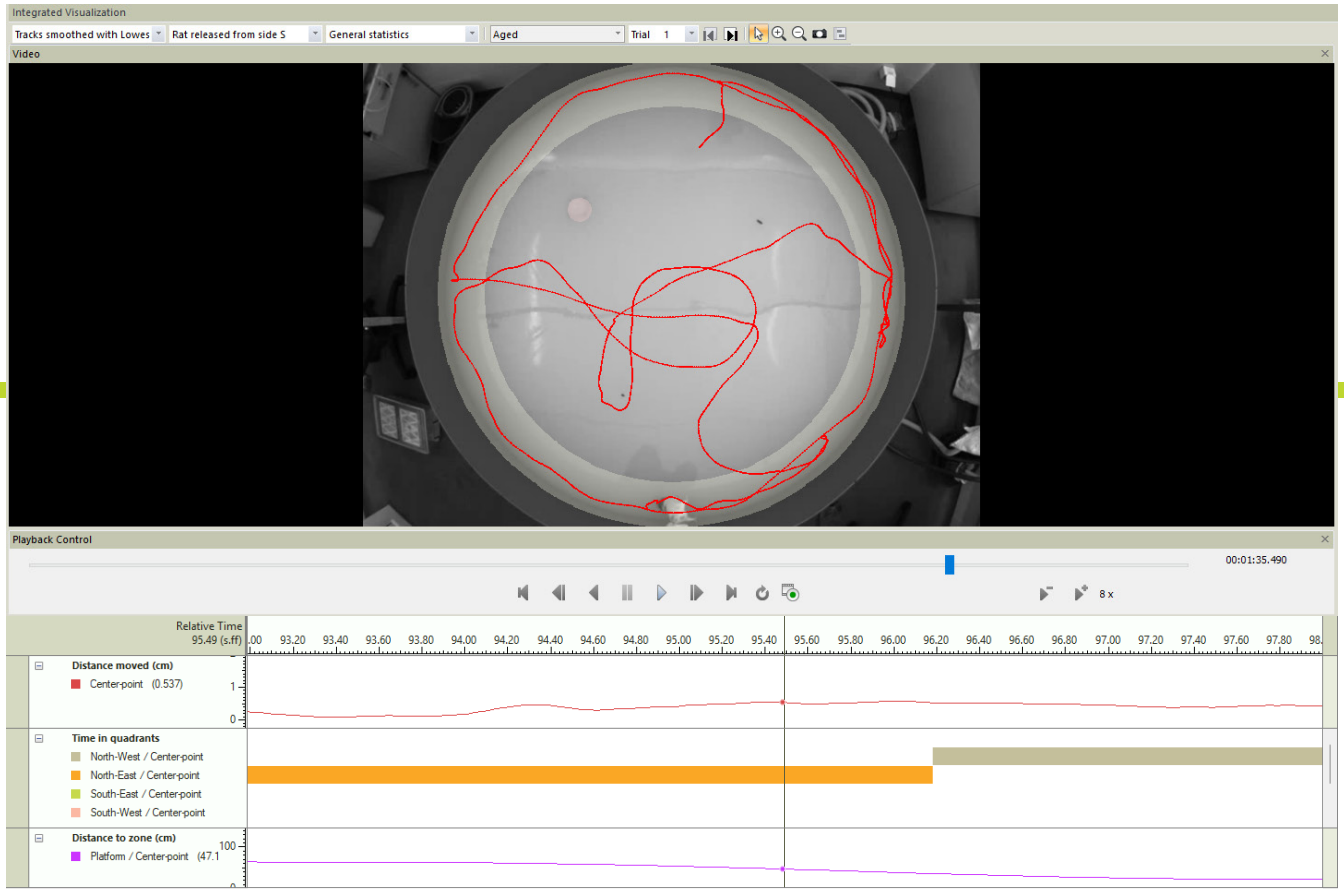
This enables accurate continuous tracking of a variety of parameters.

DEFINE ZONES OF INTEREST

With EthoVision XT, you can define custom zones directly on the video image—such as the four quadrants, platform location, wall periphery (for thigmotaxis analysis), or Whishaw's corridor (to assess path straightness).

The software automatically links data such as velocity, duration, and latency to these zones, allowing you to:

- Analyze spatial memory more precisely
- Differentiate between random and goal-directed swimming
- Have a more thorough and complete view of the animal's behavior



HOW ETHOVISION XT EMPOWERS YOUR RESEARCH

EthoVision XT is there for you to streamline your workflow and enhance the quality of your data. The software provides:

- Objective, repeatable measurements
- Efficient data processing and analysis
- Reduced experimenter bias
- Comprehensive insights into a variety of parameters.
- The most validated way to do behavioral tracking

By combining automation with flexibility, EthoVision XT empowers researchers to conduct more precise, efficient, and insightful experiments with the MWM and many others.

RESEARCH PARAMETERS

While escape latency remains the main parameter in water maze studies, other variables can provide additional insights. All these parameters and more can easily be measured and analyzed with EthoVision XT.

While escape latency remains the main parameter in water maze studies, other variables can provide additional insights. All these parameters and more can easily be measured and analyzed with EthoVision XT.

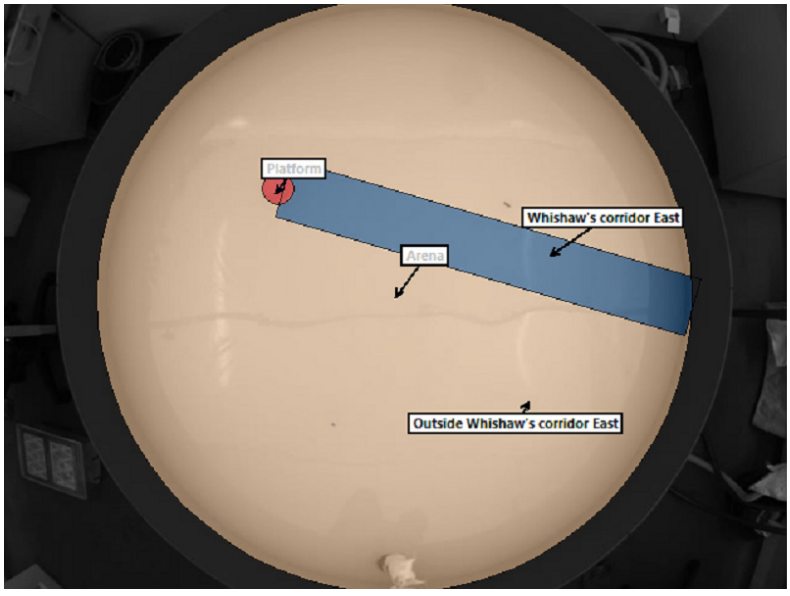
ESCAPE LATENCY

Escape latency, or latency to reach the platform, is the time it took the subject to get to the platform. All water maze studies use this parameter. It is also measured in the probe trials we then discuss how much time it took the animal to get to the specific spot where the platform used to be.

SWIM PATHS

Swim paths are indicative of the search strategy the animal is using. It might not use the external cues, for example, and simply swim in circles until it bumps into the platform. The meandering of the path towards the platform can also tell how confidently the animal is heading towards its goal.

Whishaw's index is another measure of how straight the path of the animal is from the starting point to the platform. Animals that stay within the *Whishaw's corridor* follow a straight path.



Whishaws corridor in the EthoVision XT Arena settings.

TIME SPEND IN QUADRANT/PLATFORM ZONE

The amount of time the animal spends in the correct quadrant is especially important during probe trials, as it indicates whether the animal is searching in the right place. Additionally, the numbers of crossings over the previous location of the platform are used as a reference memory indicator.

VELOCITY

Velocity reflects locomotion abilities and motivation. It is mostly used to rule out additional effects of treatment. If the treatment itself affects locomotion, this might cause differences in water maze parameters that are not caused by memory deficits. The animal might need more time to reach the platform simply because it is slower and not because of its impaired memory.

RESEARCH APPLICATIONS

The MWM has been used in many studies related to Alzheimer's research. Paired with EthoVision XT video tracking software, the water maze becomes a precise test for studying cognitive deficits and evaluating therapeutic interventions. In this next part we will explore how this setup has enabled discoveries across a variety of Alzheimer's studies.

Paired with EthoVision XT video tracking software, the water maze becomes a precise test for studying cognitive deficits and evaluating therapeutic interventions

mTOR GENE REMOVAL

The mTOR gene, central to the insulin signaling pathway, has been implicated in cognitive dysfunction associated with type 2 diabetes—a known risk factor for sporadic Alzheimer's disease (sAD). In a study by Caccamo *et al.*[1], one copy of the mTOR gene was deleted in a Tg2576 mouse model of AD. Although all subjects completed the MWM learning phase, those with mTOR gene deletion exhibited improved memory during probe trials and reduced AD-related pathology, including plaques and tangles.

GALACTOSE AS A THERAPEUTIC CANDIDATE

Insulin resistance is a key pathophysiological feature of sAD. Knezovic *et al.*[2] investigated galactose, a glucose epimer, in a rat model of sAD induced via intracerebroventricular streptozotocin (STZ-icv). After two months of oral administration, treated rats showed improved performance in the MWM, with significant gains in both learning and memory retention.

DIMETHYL FUMARATE IN AN AGE-DEPENDENT CONTEXT

Majkutewicz *et al.*[3] assessed the neuroprotective and anti-inflammatory effects of dimethyl fumarate (DMF) in STZ-icv rat models of sAD. Results indicated that older rats exhibited more pronounced cognitive deficits yet also responded more effectively to DMF treatment. These findings underscore the relevance of age as a factor in therapeutic efficacy.

ADENOSINE A2A RECEPTOR ANTAGONISM IN AD MODELS

Orr *et al.*[4] evaluated istradefylline, an adenosine A2A receptor antagonist approved for Parkinson's disease in Japan, in hAPP-J20 mice. While acquisition of the water maze task was similar across groups, istradefylline-treated mice demonstrated enhanced spatial memory during probe trials, suggesting its potential application in AD-related cognitive impairment.

GABAERGIC MODULATION AND COGNITIVE PERFORMANCE

Pilipenko *et al.*[5] explored the role of GABAergic signaling in memory function by administering muscimol and baclofen, GABA receptor agonists, to STZ-icv rats. Both treatments led to improved memory performance and reductions in neuroinflammatory markers, highlighting the therapeutic potential of GABA modulation in Alzheimer's models.

WHAT KIND OF WATER MAZE DO I NEED

The water maze is, simply put, a large round pool. However, there are many differences in size, materials, and color used for this pool.

Noldus offers pools from several manufacturers. All water maze pools we sell are perfect for video tracking experiments with EthoVision XT, and are available in a cost-efficient package deal including a camera and computer.

The water maze is a large round pool, with many variations in size and color.

SIZING AND COLOR

For example, in the five studies used as examples in this white paper [1-5], the size for mice varies between 122 and 150 cm. For rats, this varies from 150 to 180 cm. Most manufacturers deliver sizes from 100 to 180 cm.

Coloring often depends on the species used; common colors include blue, black, gray, and white.

PLATFORM

Platform sizes differ from about 10 cm to 15 cm in diameter. During the phases of the test in which the platform is hidden, it is submerged 1 to 2 cm below surface level.

Some manufacturers offer automatic platforms, which can be controlled with EthoVision XT video tracking software. This way, the platform can be lifted or collapsed while tests are in progress. Using up to four platforms allows you to switch out the quadrant position of the platform (by lowering the others) without your hands touching the water.

WATER COLOR AND TEMPERATURE

Temperature can be very important in a Morris water maze test.

Many researchers feel that rats like swimming better than mice, making them easier to handle in this test but less motivated to find the platform. Some also say that rats don't like colder water. As a result, water temperature can be a very important factor for the results of the test.

In the research examples of this document, water temperature varied between 20 and 25 degrees Celsius, with a variation of one or two degrees.

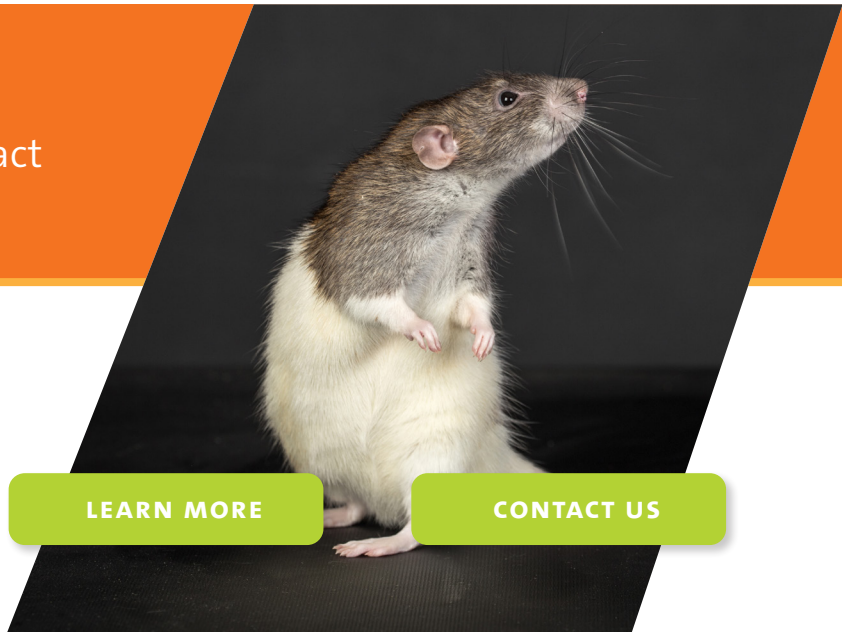
In addition to temperature, the water color can be of importance. Especially in white pools, the water is often made opaque white. This can improve contrast with the subject for video tracking and, as Caccamo *et al.*[1] mentions, it prevents the animal from seeing the platform itself. The water can be made white with non-toxic paint [1,4] or milk powder.

CUES

Because this is a spatial navigation test and the animal is supposed to learn the location of the platform from various starting positions, visual cues are important. These can either be placed within the walls of the maze [3] or elsewhere in the room [1,4].

We are here to help you out.

Keep reading to find out more or contact us now to discuss your research goals.



Continue reading to learn more about:

- What is the best maze for you
- Various benefits of EthoVision XT
- How Noldus assists you from the start to finish

LEARN MORE

CONTACT US

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