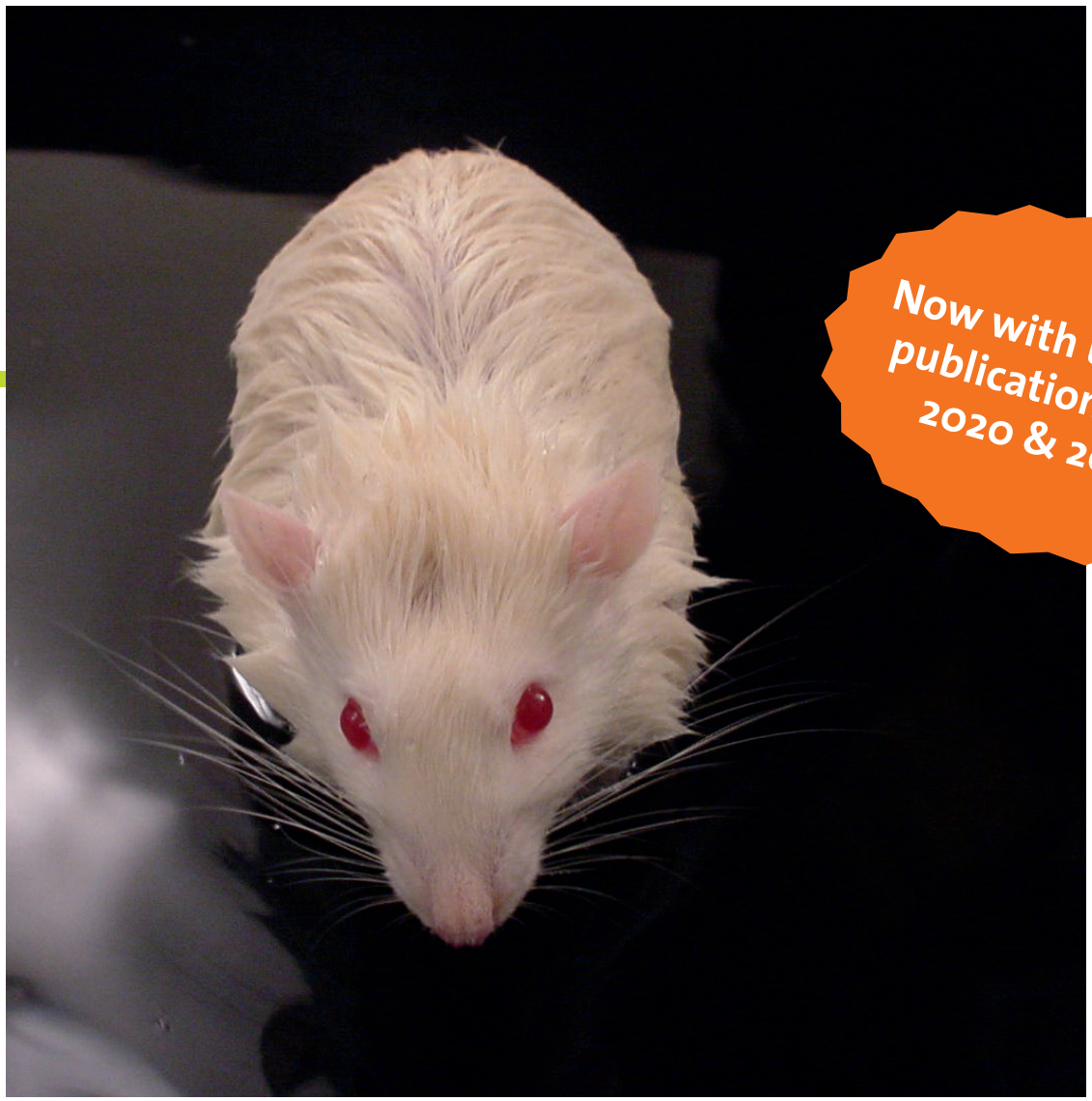


Now with updated publication list of 2020 & 2021!

Water maze testing and Alzheimer's

How EthoVision XT can benefit your research

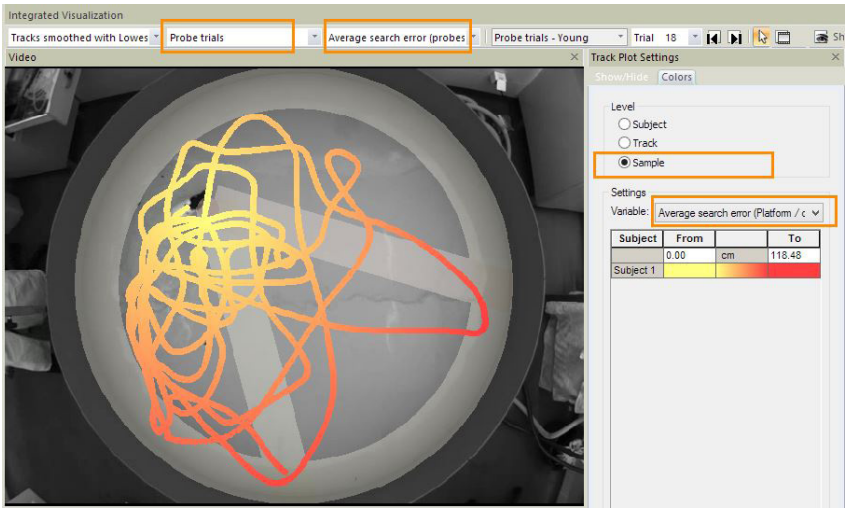
A white paper by Noldus Information Technology

WATER MAZE TESTING AND ALZHEIMER'S RESEARCH

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

The Morris water maze or navigation task is named after its maker: *Richard Morris*. He first developed the test in 1981, and now it is one of the most used tests for spatial learning and memory in behavioral neuroscience.

During the test, a rat or mouse learns to locate a platform hidden just beneath the water surface of a relatively large pool. Normally, animals learn to locate the platform at an increasingly faster rate during consecutive trials depending on their spatial learning abilities.



Most experiments also include a probe trial, in which the platform is missing. Is the animal searching for the platform in its previous location? If so, this tells us something about its memory.

Alzheimer's disease (AD) is the most common form of dementia, and it is also one of the most common neurodegenerative diseases. AD comes with memory impairments, and as a result many of the neurobehavioral studies done on AD involve the water maze test.

RESEARCH EXAMPLES

In this white paper, a sample of recent studies illustrates how the Morris water maze task is used for research on possible AD therapeutics, and how video tracking methodology is incorporated in these experiments.

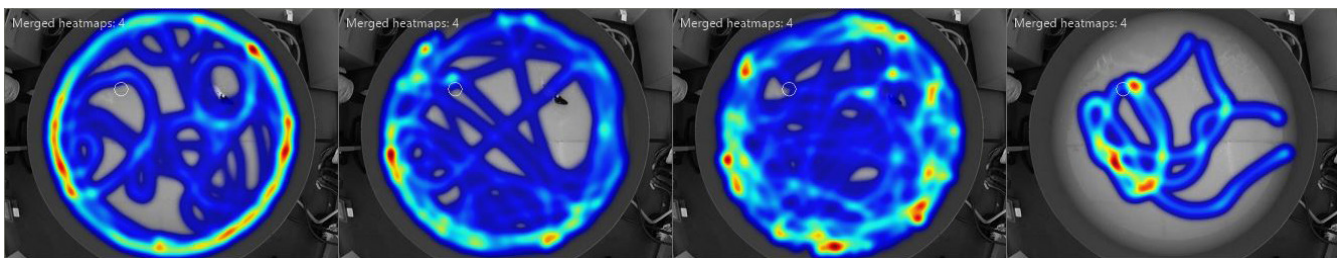
ALZHEIMER'S RISK FACTORS AND THERAPEUTIC TARGETS

In these studies, several AD risk factors are mentioned. One major focus is insulin resistance and diabetes [1,2,3], as there is an increasing amount of evidence suggesting this is closely linked the memory problems AD-patients experience.

Another target is adenosine, which is naturally present in every cell of our body and acts as an inhibiting neurotransmitter in our brain. Orr *et al.* [4] investigate the effects of adenosine A2A receptor blockers on memory formation in mice models of AD.

Neuroinflammation also has a key role in AD pathology. GABA is another neurotransmitter that is thought to have a role in neuroinflammation and memory formation, and thus the GABA system might also be an interesting target for AD therapeutics. Pilipenko *et al.* [5] investigated two GABA receptor agonists: muscimol and baclofen.

All of the studies referenced to in this white paper have used Etho-Vision XT video tracking for their Morris water maze experiments.



WATER MAZE SPECIFICS

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

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. We can also build a custom pool to your specifications. 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.

SIZING AND COLOR

For example, in the five studies used as examples in this white paper [1,2,3,4,5], 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.

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

WATER COLOR AND TEMPERATURE

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].

WATER MAZE TRIALS

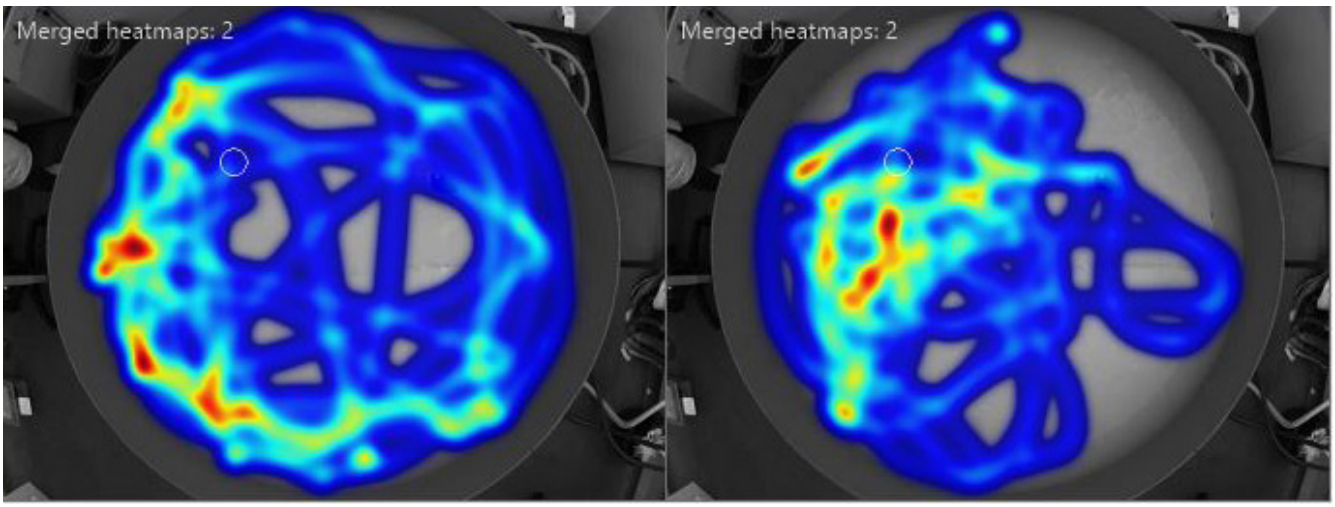
The water maze test typically involves several days of learning trials, followed by a probe trial.

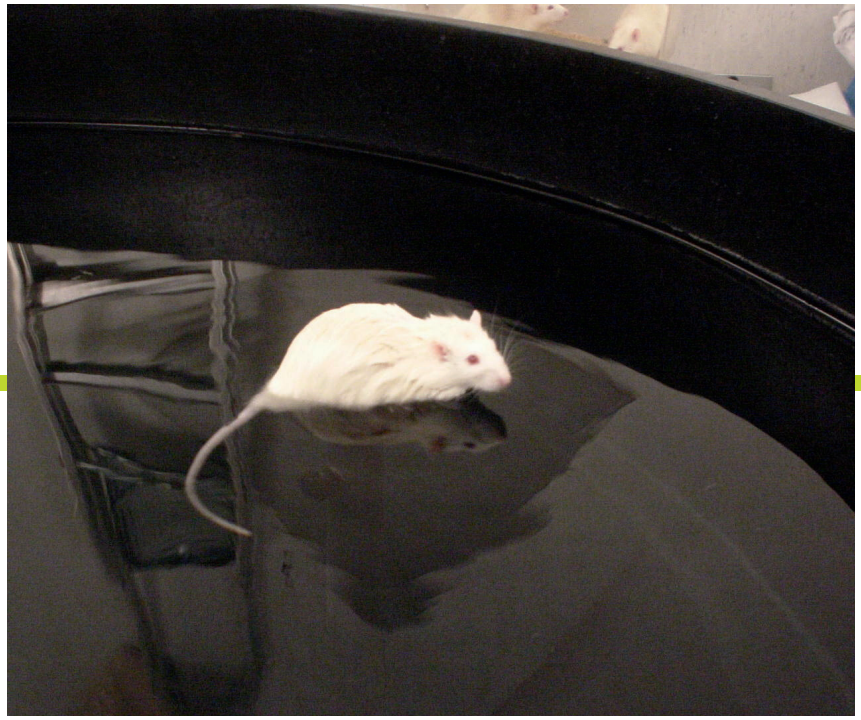
LEARNING PHASE TRIALS

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 [1234] 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.





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).

VIDEO TRACKING IN THE WATER MAZE

Arguably, the most important parameter in the water maze test is escape latency. In other words, escape latency is a measure of how quickly the subject reaches the platform.

While this might seem easy to measure by hand (using just a stopwatch), it is much more accurately done with video tracking, as EthoVision XT can objectively tell when the animal reached the platform exactly and in the exact same way for every individual subject.

HOW VIDEO TRACKING WORKS

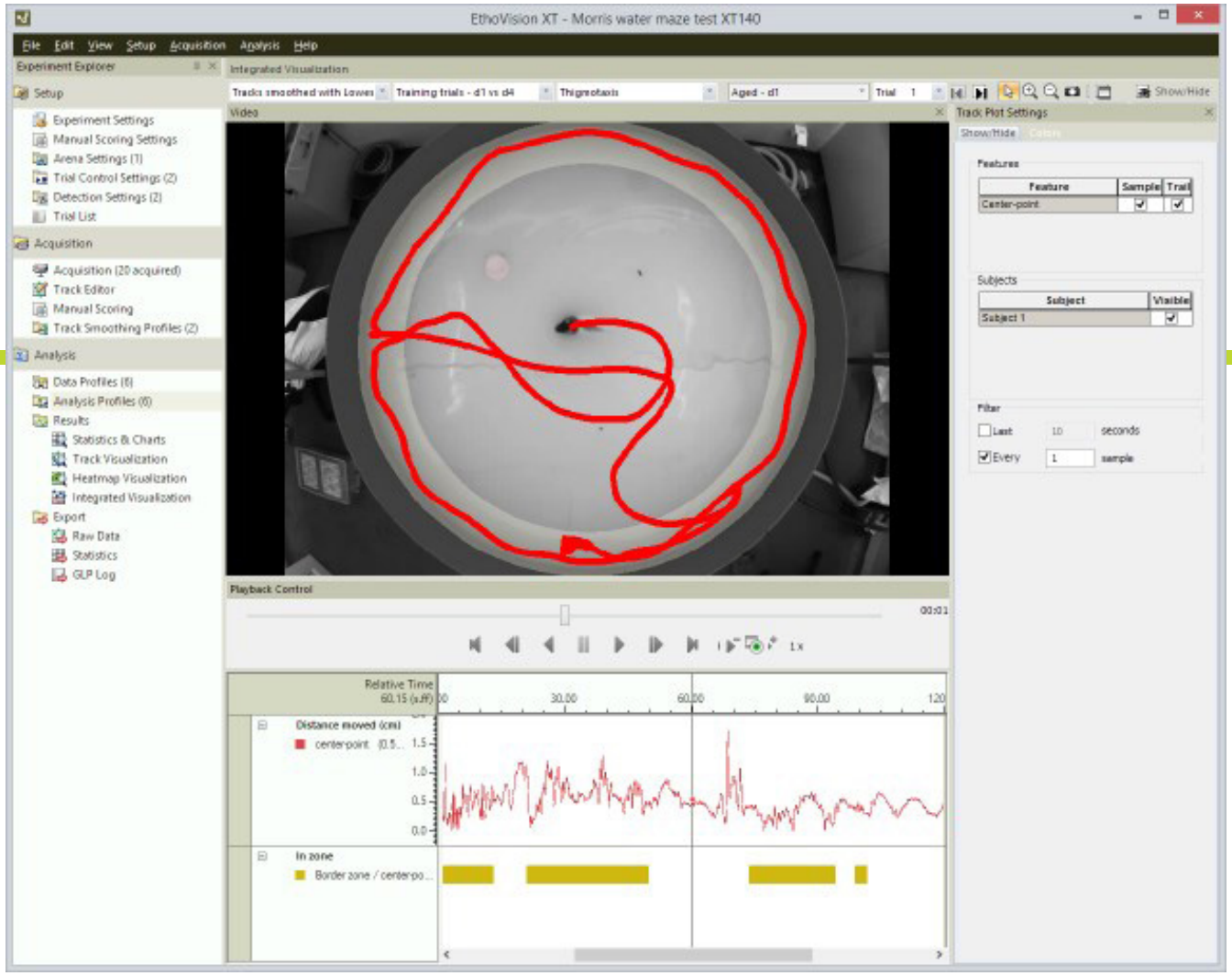
A camera is mounted above the water maze pool, and this camera is connected to a computer running EthoVision XT video tracking software. The video stream can be tracked live, and pre-recorded videos can be tracked and analyzed at a later time.

EthoVision XT detects the animal and tracks its movement and activity as it swims through the pool. This way, it knows where the subject is at all times during the trial, where it spends most of its time, how fast it swims, and what its swim path is.

AREAS OF INTEREST

Because EthoVision XT allows you to 'draw' zones of interest in the video image of the pool, you can easily indicate each quadrant and platform location as well as additional zones such as wall perimeter (e.g. to measure thigmotaxis) or Whishaw's corridor.

This allows EthoVision XT to automatically relate distances, velocity, durations, and latencies to these areas of interest. All of these parameters and areas can be used for data selection and analysis within EthoVision XT.


EthoVision XT - Morris water maze test XT140
File Edit View Setup Acquisition Analysis Help
Experiment Explorer
Setup
Experiment Settings
Manual Scoring Settings
Arena Settings (1)
Trial Control Settings (2)
Detection Settings (2)
Trial List
Acquisition
Acquisition (20 acquired)
Track Editor
Manual Scoring
Track Smoothing Profiles (2)
Analysis
Data Profiles (6)
Analysis Profiles (6)
Results
Statistics & Charts
Track Visualization
Heatmap Visualization
Integrated Visualization
Export
Raw Data
Statistics
GLP Log
Integrated Visualization
Tracks smoothed with Lowes
Training trials - d1 vs d4
Thigmotaxis
Aged - d1
Trial 1
Show/Hide
Video
Track Plot Settings
Show/Hide
Colors
Features
Feature
Sample
Trail
Center-point
Subjects
Subject
Visible
Subject 1
Filter
Last
10
seconds
Every
1
sample
Playback Control
1x
Relative Time
60.15 (s.dff)
30.00
60.00
90.00
120
Distance moved (cm)
center-point (0.5...
1.5
1.0
0.5
0.0
In zone
Border zone / center-po...

RESEARCH PARAMETERS

Escape latency is the most commonly used parameter, but there are also other more important variables in the Morris water maze test.

ESCAPE LATENCY

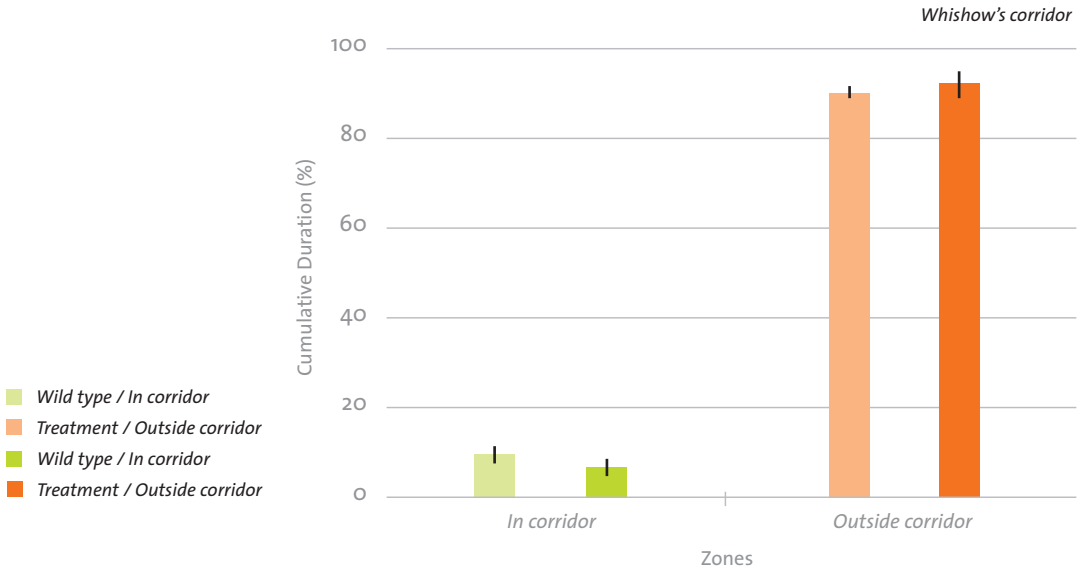
Escape latency, or latency to reach the platform, is the time it took the subject to get to the platform. All water maze studies use this parameter. It is also measured in the probe trials we then discuss how much time it took the animal to get to the specific spot where the platform used to be.

Time in correct 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 is used as a reference memory indicator.

MISTAKES

Knezovic *et al.* [2] defines the number of mistakes as incorrect quadrant entries.

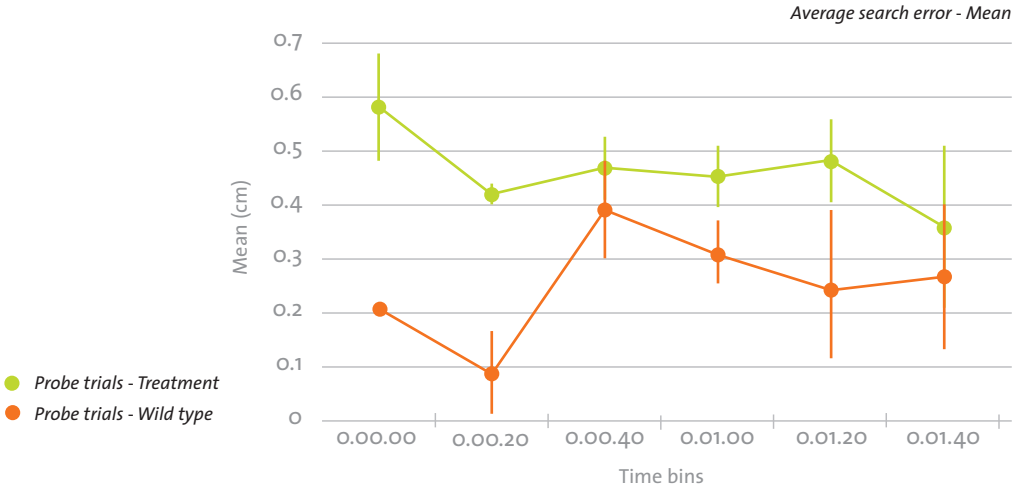


SWIM PATHS

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

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.



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.

All these parameters are easily measured and analyzed by EthoVision XT. Using zone-based tracking, all variables such as speed, distance, and location can be easily related to the quadrant or to Whishaw's corridor.

STUDY RESULTS

mTOR GENE REMOVAL

Type two diabetes increases the risk of sporadic Alzheimer's disease (sAD), so Caccamo *et al.* [1] focused on the mTOR gene, which plays a crucial role in the insulin signaling pathway. Using a cross-breeding strategy, they removed one copy of the mTOR gene in a Tg2576 mouse model of AD.

All mice learned to locate the platform in five days, but their learning pace differed. During the probe trial, it became obvious that gene deletion rescued the memory deficits of the 'sAD mice.' mTOR removal improved cognition and other pathological hallmarks (plaques and tangles).

GALACTOSE TREATMENT

Insulin resistance is believed to be at the pathophysiological core of sAD. Knezovic *et al.* [2] investigated whether galactose, an epimer of glucose, could alleviate memory impairment in a rat model of sAD (intracerebroventricular streptozotocin administration, or STZ-icv model). Oral administration for 2 months normalized the impaired learning and memory functions. Learning ability in these rats was greatly improved as well as memory retention.

DMF TREATMENT

Majkutewicz *et al.* [3] also looked at insulin resistance and investigated the age-dependent effects of dimethyl fumarate (DMF), an anti-inflammatory, antioxidative, and neuroprotective drug. They also used STZ-icv rats as a model for sAD. The researchers found that spatial memory and neurodegeneration is more severe in aged STZ rats, but DMF therapy is also more effective.

ADENOSINE BLOCKERS

Orr *et al.* [4] used hAPP-J20 mice as a model of AD to test whether the adenosine A2A receptor antagonist istradefylline, an improved drug in Japan for Parkinson's disease, would also alleviate memory problems in patients with AD.

While learning was the same in 'AD-mice' as in control animals, low dose treatment with istradefylline did enhance spatial memory in hAPP-J20 mice, suggesting it might counteract memory problems in AD patients as well.

GABA BLOCKERS

Pilipenko *et al.* [5] investigated the role of GABA memory formation in STZ-icv rats by treating these 'AD-rats' with GABA receptor agonists: muscimol and baclofen. Both substances exerted memory-enhancing (and anti-inflammatory) effects.

13 — *The water maze test*

REFERENCES

1. Caccamo, A.; Belfiore, R.; Oddo, S. (2018). Genetically reducing mTOR signaling rescues central insulin dysregulation in a mouse model of Alzheimer's disease. *Neurobiology of Aging*, doi: 10.1016/j.neurobiolaging.2018.03.032.
2. Knezovic, A.; Osmanovic Barilar, J.; Babic, A.; Bagaric,R.; Farkas, V.; Riederer, P.; Salkovic-Petrisic, M. (2018). Glucagon-like peptide-1 mediates effects of oral galactose in streptozotocin-induced rat model of sporadic Alzheimer's disease. *Neuropharmacology*, **135**, 48-62.
3. Majkutewicz, I.; Kurowska, E.; Podlacha, M.; Myslinska, D.; Grembecka, B.; Rucinski, J.; Pierzynowska, K.; Wrona, D. (2018). Age-dependent effects of dimethyl fumarate on cognitive and neuropatho"-logical features in the streptozotocin-induced rat model of Alzheimer's dise*ase*. *Brain Research*, **1686**, 19-33.
4. Orr, A.G.; Lo, I.; Schumacher, H.; Ho, K.; Gill, M.; Guo, W.; Kim, D.H.; Knox, A.; Saito, T.; Saido, T.C.; Simms. J.; Toddes, C.; Wang, X.; Yu, G.-Q.; Mucke, L. (2018). Istradefylline reduces memory deficits in aging mice with amyloid pathology. *Neurobiology of Disease*, **110**, 29-36.
5. Pilipenko, V.; Narbute, K.; Beitnere, U.; Rumaks, J.; Pupure, J.; Jansone, B.; Klusa, V. (2018). Very low doses of muscimol and baclofen ameliorate cognitive deficits and regulate protein expression in the brain of a rat model of streptozocininduced Alzheimer's disease. *European Journal of Pharmacology*, **818**, 382-399.

The example project for the EthoVision XT data and images were kindly provided by Dr. Istvan Hernadi from the Department of Experimental Neurobiology from the University of Pecs, in Pecs, Hungary.

UPDATED LIST OF PUBLICATIONS

- Anderson, J.E.; Trujillo, M.; McElroy, T.; Groves, T.; Alexander, T.; Kiffer, F.; Allen, A.R. (2020). Early effects of Cyclophosphamide, Methotrexate, and 5-Fluorouracil on neuronal morphology and hippocampal-dependent behavior in a murine model. *Toxicological Sciences*, **173(1)**, 156-170.
- Belaya, I.; Ivanova, M.; Sorvari, A.; Ilicic, M.; Loppi, S.; Koivisto, H.; Varricchio, A.; Tikkanen, H.; Walker, F.R.; Atalay, M.; Malm, T.; Grubman, A.; Tanila, H.; Kanninen, K.M. (2020). Astrocyte remodeling in the beneficial effects of long-term voluntary exercise in Alzheimer's disease. *Journal of Neuroinflammation*, **17**, 271.
- Bellantuono, I.; Cabo, R. de; Ehninger, D.; Di Germanio, C.; Lawrie, A.; Miller, J.; Mitchell, S. J.; Navas-Enamorado, I.; Potter, P. K.; Tchkonia, T.; Trejo, J. L.; Lamming, D. W. (2020). A toolbox for the longitudinal assessment of healthspan in aging mice. *Nature Protocols*, **15**, 540-574.
- Chen, Q.; Sun, K.-P.; Huang, J.-S.; Wang, Z.-C.; Hong, Z.-N. (2020). Resveratrol attenuates neuroinflammation after deep hypothermia with circulatory arrest in rats. *Brain Research Bulletin*, **155**, 145-154.
- Fernandez-Valenzuela, J. J.; Sanchez-Varo, R.; Muñoz-Castro, C.; De Castro, V.; Sanchez-Mejias, E.; Navarro, V.; Jimenez, S.; Nuñez-Diaz, C.; Gomez-Arboledas, A.; Moreno-Gonzalez, I.; Vizuete, M.; Davila, J.C.; Vitorica, J.; Gutierrez, A. (2020). Enhancing microtubule stabilization rescues cognitive deficits and ameliorates pathological phenotype in an amyloidogenic Alzheimer's disease model. *Scientific Reports*, **10**, 14776.
- Calvo-Flores Guzmán, B.; Chaffey, T.E.; Palpagama, T.H.; Waters, S.; Boix, J.; Tate, W.P.; Peppercorn, K.; Dragunow, M.; Waldvogel, H.J.; 1, Faull, R.L.M.; Kwakowsky, A. (2020). The interplay between Beta-Amyloid 1–42 (Aβ1–42)-induced hippocampal inflammatory response, p-tau, vascular pathology, and their synergistic contributions to neuronal death and behavioral deficits. *Frontiers in Molecular Neuroscience*, **13**, 522073.
- Gulbranson, D.R.; Ho, K.; Yu, G.-Q.; Yu, X.; Das, M.; Shao, E.; Kim, D.; Zhang, W.J.; Choudhary, K.; Thomas, R.; Mucke, L. (2021). Phenotypic differences between the Alzheimer's disease-related hAPP-J20 model and heterozygous Zbtb20 knockout mice. *eNeuro*, 10.1523.
- Johnson, E.C.B.; Ho, K.; Yu, G.-Q.; Das, M.; Sanchez, P.E.; Djukic, B.; Lopez, I.; Yu, X.; Gill, M.; Zhang, W.; Paz, J.T.; Palop, J.J.; Mucke, L. (2020). Behavioral and neural network abnormalities in human APP transgenic mice resemble those of App knock-in mice and are modulated by familial Alzheimer's disease mutations but not by inhibition of BACE1. *Molecular Neurodegeneration*, **15**, 53.
- Lina, F.-Y.; Lina, Y.-F.; Lina, Y.-S.; Yang, C.-M.; Wang, C.-C.; Hsiao, Y.-H. (2020). Relative D3 vitamin deficiency and consequent cognitive impairment in an animal model of Alzheimer's disease: Potential involvement of collapsing response mediator protein-2. *Neuropharmacology*, **164**, 107910.

- Müller, L.; Power Guerra, N.; Stenzel, J.; Rühlmann, C.; Lindner, T.; Krause, B.J.; Vollmar, B.; Teipel, S.; Kuhla, A. (2021). Long-Term Caloric Restriction Attenuates β-Amyloid Neuropathology and Is Accompanied by Autophagy in APPswe/PS1deltaE9 Mice. *Nutrients*, **13**, 985.
- Pan, Y.; Zhang, Y.; Liu, N.; Lu, W.; Yang, J.; Li, Y.; Liu, Z.; Wei, Y.; Lou, Y.; Kong, J. (2021). Vitamin D Attenuates Alzheimer-like Pathology Induced by Okadaic Acid. *ACS Chemical Neuroscience,* **12(8)**, 1343-1350.
- Szatmari, E.; Moran, C.; Cohen, S.; Jacob, A.; Parra-Bueno, P.; Kamasawa, N.; Guerrero-Given, D.; Klein, M.; Stackman, R.; Yasuda, R. (2020). ADAP1/Centaurin-α1 negatively regulates dendritic spine function and memory formation in the hippocampus. *eNeuro*, 10.1523.
- Upreti, C.; Woodruff, C.M.; Zhang, X.-L.; Yim, M.J.; Zhou, Z.-Y.; Pagano, A.M.; Rehanian, D.S.; Yin, D.; Kandel, E.R.; Stanton, P.K.; Nicholls, R.E. (2021). Loss of retinoid X receptor gamma subunit impairs group 1 mGluR mediated electrophysiological responses and group 1 mGluR dependent behaviors. *Scientific Reports*, **11**:5552.
- Wu, J.; Bie, B.; Foss, J.F.; Naguib, M. (2020). Amyloid Fibril–Induced Astrocytic Glutamate Transporter Disruption Contributes to Complement C1q-Mediated Microglial Pruning of Glutamatergic Synapses. *Molecular Neurobiology*, 10.1007.
- Zhao, Y.; Kiss, T.; DelFavero, J.; Li, L.; Li, X.; Zheng, L.; Wang, J.; Jiang, C.; Shi, J.; Ungvari, Z.; Csiszar, A.; Zhang, X.A. (2020). CD82-TRPM7-Numb signaling mediates age-related cognitive impairment. *GeroSience*, **42(2)**, 595–611.

INTERNATIONAL HEADQUARTERS

Noldus Information Technology bv
Wageningen, The Netherlands
Phone: +31-317-473300
Fax: +31-317-424496
E-mail: info@noldus.nl

NORTH AMERICAN HEADQUARTERS

Noldus Information Technology Inc.
Leesburg, VA, USA
Phone: +1-703-771-0440
Toll-free: 1-800-355-9541
Fax: +1-703-771-0441
E-mail: info@noldus.com

REPRESENTATION

We are also represented by a worldwide network of distributors and regional offices. Visit our website for contact information.

WWW.NOLDUS.COM