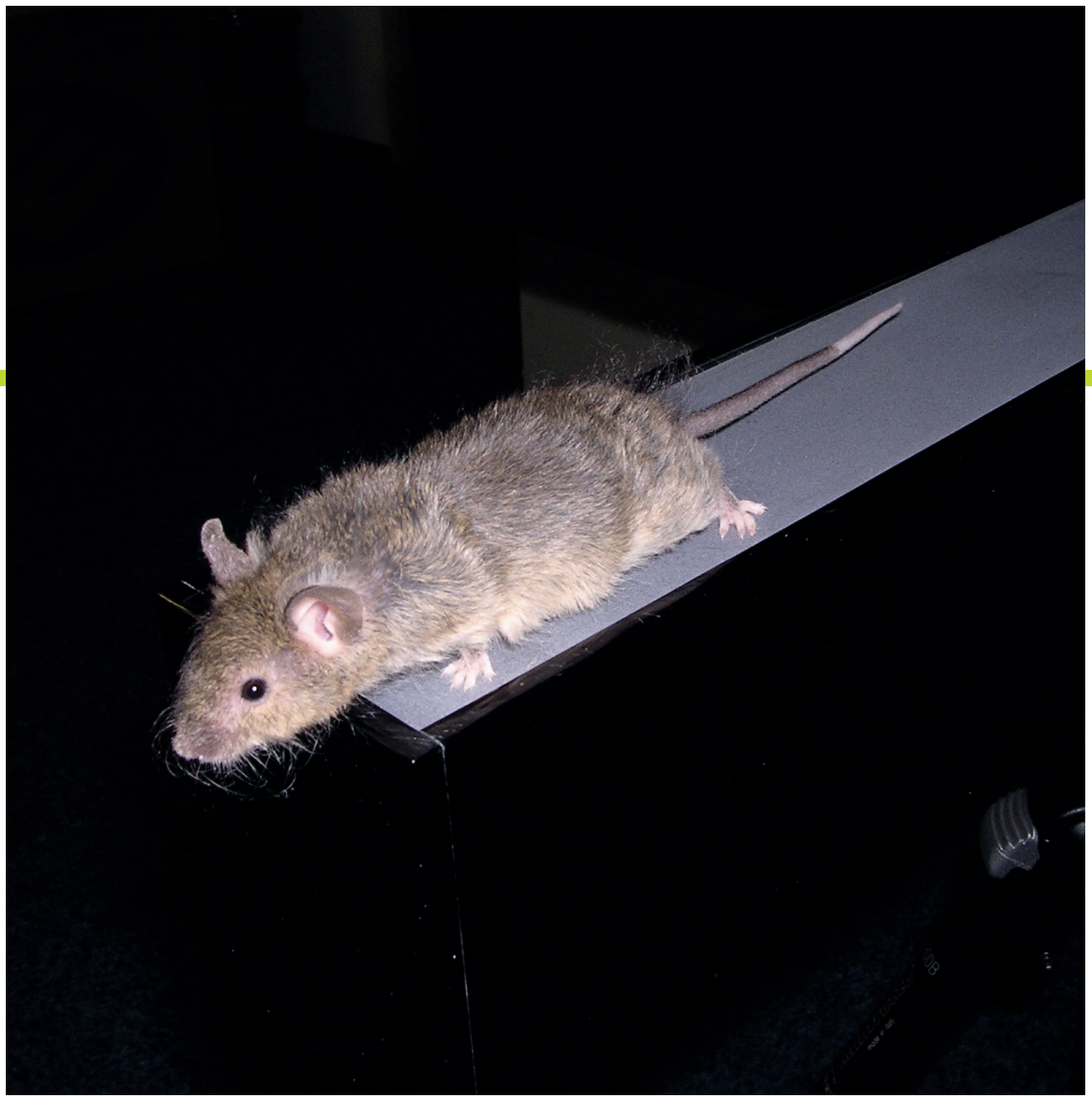


The elevated plus maze

How EthoVision XT can benefit your research

A white paper by Noldus Information Technology

INTRODUCTION

The maze consists of two open arms and two closed (wall sheltered) arms, and relies upon the animal's natural tendency to stay in enclosed spaces.

One of the most well established, and robust, behavioral tests in behavioral neuroscience is the elevated plus maze. It is designed as a simple screening test for anxiety-like behavior in rats and mice. The maze has a simple, yet effective, design consisting of two open arms and two closed (wall sheltered) arms, which are raised from the ground. The test relies on thigmotaxis; the tendency to be close to vertical surfaces, and on the innate unconditioned fear rodents have for open spaces and heights. This results in animals that present a higher level of anxiety spending more time in the closed arms while more time on the open arms relates to lower anxiety-like behavior.

This paradigm is used in many studies that focus on the basics of anxiety disorders [1]. This is also applied to study the effects of certain treatments and/or pathogenesis of cognitive and/or (neuro)psychiatric disorders such as Alzheimer's disease [2], autism [3] and schizophrenia [4]. The elevated plus maze is furthermore used in neurodevelopmental studies [5], but also to study addiction [4] and PTSD [6]. It has become a standard test for acute anxiety assessment since the introduction by Pellow *et al.* in 1985, who initially used it to study the selective identification of anxiolytic and anxiogenic drug effects in rats [7].

VIDEO ANALYSIS AND THE ELEVATED PLUS MAZE

Video tracking is a way to automate your research that works very well for the elevated plus maze test. The advantages include more efficient research, and reliable and objective results. The studies mentioned in this paper are all recently published and all used EthoVision video tracking to automate their elevated plus maze test.

EXAMPLES OF STUDIES

KOCAHAN ET AL. [1]

Used the elevated plus maze to study anxiety and activity parameters in rats in order to explore sex differences to gain a better understanding of different animals models of psychiatric disorders.

RIDDLE ET AL. [2]

Investigated the effects of physical exercise on cognitive function in a transgenic Alzheimer's disease mouse model as increased physical exercise could improve cognitive function and reduces pathology associated with Alzheimer's disease (AD).

AFROZ ET AL. [3]

Linked the gut microbiome to autism behavior, which is characterized by the abnormal development of the central nervous system. They found that a parental high-salt diet consumption was strongly associated with offspring that shows autism-like behavioral abnormalities via changes in the gut microbiome.

HUR ET AL. [4]

Investigated two strongly linked topics: Schizophrenia and addictive behavior. They specifically looked at Methoxphenidine, which is a dissociative-based novel psychoactive designer drug and has been found to cause schizophrenia-related symptoms in mice.

EL BOUKHARI ET AL. [5]

Studied the effects of a lesion in the thalamic reticular nucleus. Early damage to this area will cause major disturbances to the development and the functioning of the prefrontal cortex and mediodorsal nucleus; important during brain maturation.

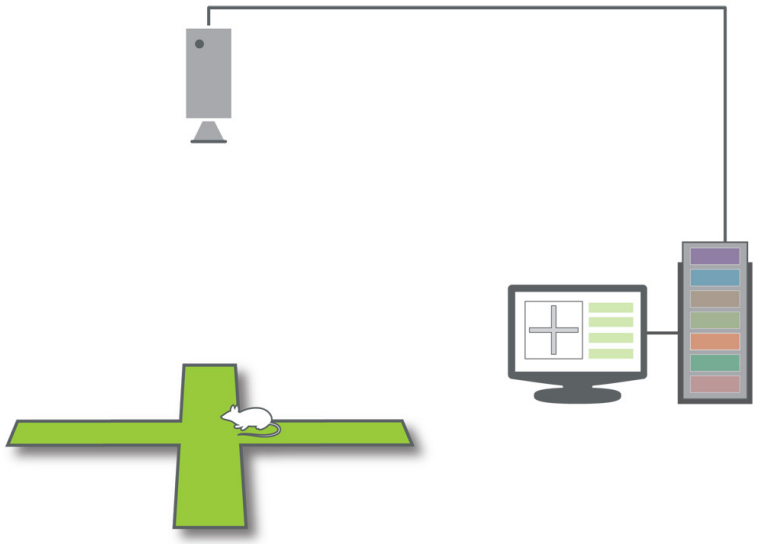
ALBRECHT ET AL. [6]

Studied a rat model of PTSD, where they used behavioral profiling as a translational approach to increase the validity of animal models of PTSD.

MATERIALS

The elevated plus maze package contains fully functional EthoVision XT software, an elevated plus maze with camera mount, a computer, and a video camera

Dimensions of the maze depend upon the animal being used. Versions designed for rats typically have arms that are 50 cm long and 10 cm wide, leaving a 10 cm by 10 cm central square that often serves as a starting area. The walls sheltering the closed arms are 30 cm or 40 cm in height. For mice, the dimensions of the maze are often smaller, for example 30 cm x 6 cm. For both rodent species, the plus maze is elevated at least 50 cm above the floor. A camera can be mounted directly above the maze so that the animal can be video tracked as it moves through each arm. To facilitate easy cleaning, the maze is usually made out of plexiglass.



METHODS

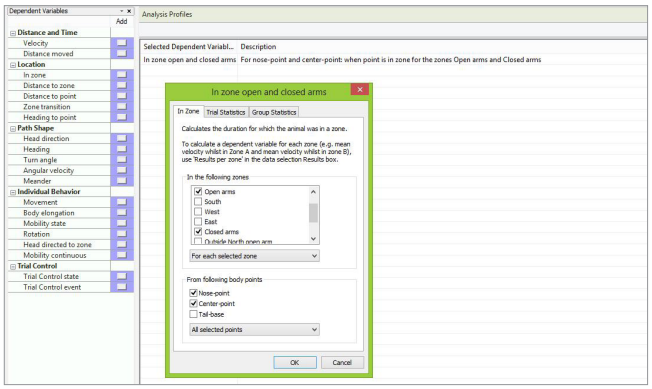
Starting an elevated plus maze firstly requires habituation to the testing room, and when animals are housed socially preferably the animals are also socially separated 5-10 minutes before starting the test. After habituation the test can be started by individually placing the animal in the maze. The most generally accepted way of doing this is placing the mouse or rat in the central square of the maze. Some researchers place their animals facing the open arm, and others let them face the closed arm, the important thing is that this is kept consistent in the protocol across the entire experiment. A trial typically lasts 5 minutes. During this time, the animal is video tracked, automatically monitoring the time spent in each area of the maze. In addition, behaviors such as head dipping and rearing can be scored. To add additional details to the data, the nose point and tail base can be tracked in addition to the center point of the animal.

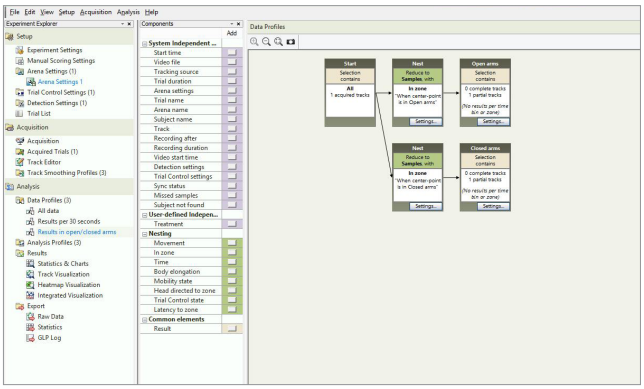
EthoVision XT includes a project template for the elevated plus maze so you can get your experiment up and running quickly.

THE ADVANTAGES OF VIDEO ANALYSIS

With EthoVision XT you can define all areas of the arena: the closed arms, open arms, and central square. This way latency to enter an open arm, time spent in each of the arms, and other variables related to zones are automatically measured. Ethovision XT can detect and score additional behaviors such as head dipping and rearing as well, but it is also possible to use the manual event recorder that is included in the software. These observations can all be integrated with the rest of your data.

With EthoVision XT you can define the areas of interest in the arena so that variables can be linked to these zones.





EthoVision XT includes a project template for the elevated plus maze so you can get your experiment up and running quickly. There are several ways to proceed from here. Either you record your videos and track them later, or you track live and record the videos at the same time for later reference. Trial Control allows you to set conditions for the automatic start and stop of each trial. For example, you can set EthoVision XT to start tracking automatically as soon as the animal is detected in the arena and stop 5 minutes after that.

If you are using live tracking and have a large number of trials to run, it is possible to set the starting and stopping conditions for each trial (just like Trial Control) and an inter-trial interval. This gives you time to remove the animal, clean up, and place the next subject, avoiding having to go back to your computer to start each trial after you have placed the animal. After activation of this feature, your focus can be fully directed at the experiment. If you choose to record all the videos beforehand, and track them later, you can perform batch acquisition and even batch analysis. This way all the videos are tracked and analyzed sequentially according to your selection of data and variables. One push of a button, and the software takes care of the rest, ensuring an extremely time-efficient, and reliable, analysis of your experiment.

COMPLETE PACKAGES

For a complete set-up, take advantage of the Noldus package deals. The elevated plus maze package contains fully functional EthoVision XT software, an elevated plus maze with camera mount, a computer and a video camera.

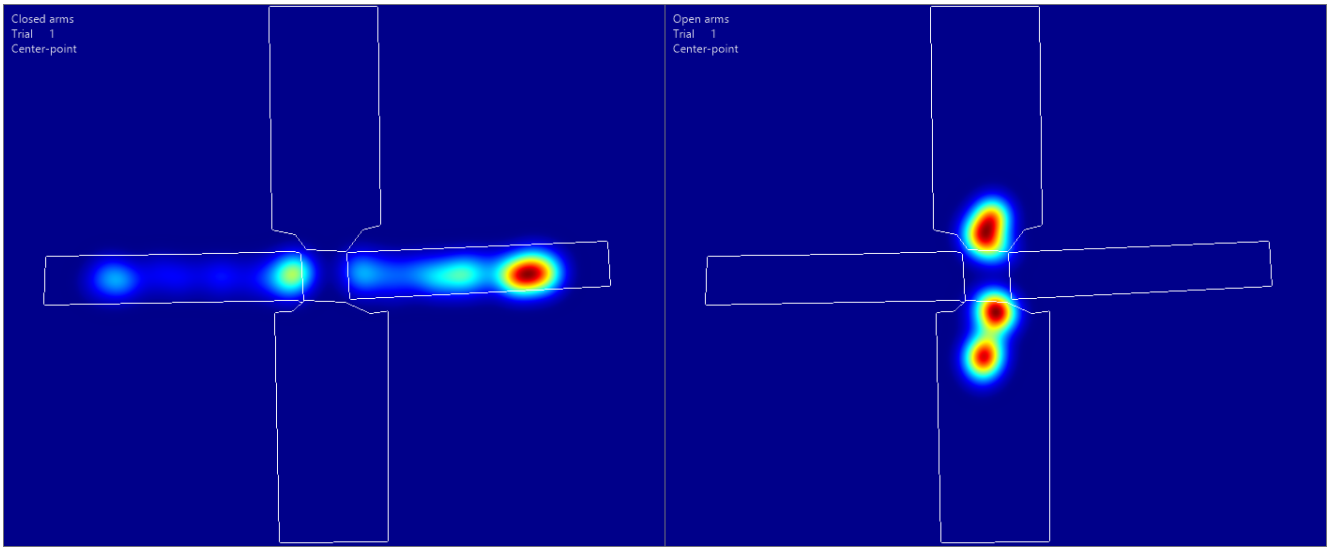
DATA

Three points of the animal are tracked: nose point, centre point, and tail base.

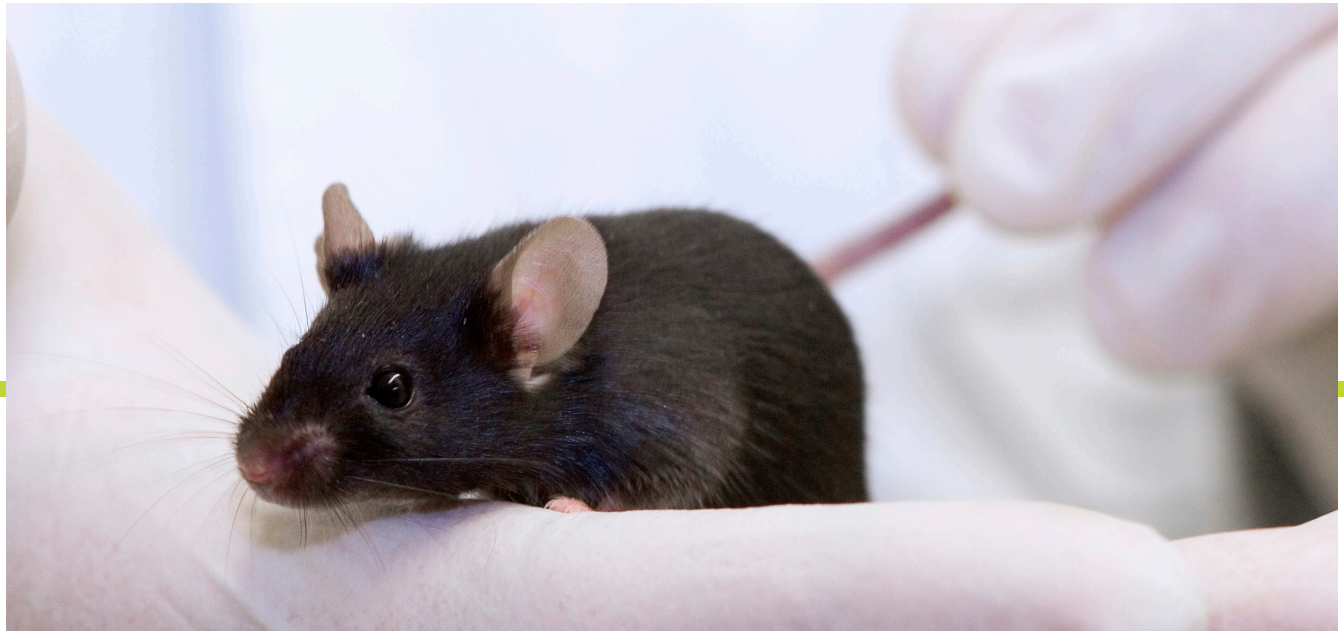
What researchers are looking to get out of these experiments is the relative time spent in each of the arms. More relative time spent (or distance travelled) in the closed arms versus the open arms indicates more anxiety-like behavior. Therefore the open arm ratio is the most validated, and used, outcome parameter that gives an indication of anxiety-like behavior in the elevated plus maze. This is calculated by dividing the total time spent on the open arms, by the total time spent op open and closed arms: O/(O+C) where O= total time spent in the open arms and C= total time spent in the closed arms. This thus results in a higher open arm ratio for animals that present lower anxiety-like behavior, and vice-versa. Additionally, latency to enter the open arm for the first time and total number of entries for each arm are also of interest and can provide more information of the animal's behavior in the maze, which is similar for parameters such as rearing or head dipping.

VIDEO TRACKING: DATA AND ANALYSIS

EthoVision XT acquires data by collecting information about the animal's whereabouts in each video frame. Three points of the animal are tracked: nose point, centre point, and tail base. This is essential so that the system



Data and analysis profiles allow you to select parts of your data and the variables you want calculated. In this example the data profiles separates data for the open arms and the closed arms. The analysis profile selects time spent in open and closed arms, in which the animal is seen as in an open or closed arm as soon as both his nose point and center point are detected there.



can accurately discriminate between an animal entering an open arm, or only poking its nose around the corner. This is combined with zone definition to automatically calculate percentage of time spent in open versus closed arms, total time spent in each arm, latency to enter one of the open arms, total entries into each one of the arms, time spent in the centre square, total time spent rearing and total number of head dips.

EthoVision XT offers several statistical methods to analyze your data, and different ways to present the results. The integrated visualization gives a dynamic view of your results, in sync with the video of the trial. Statistics are presented for individuals and groups, in tables and charts, so you can easily compare be tween groups and/or phases in your study. In addition, you can create and easily export high resolution heatmaps, density plots that give an immediate impression of how your animals spent their time, and how this compares between groups.

RESULTS IN RESEARCH

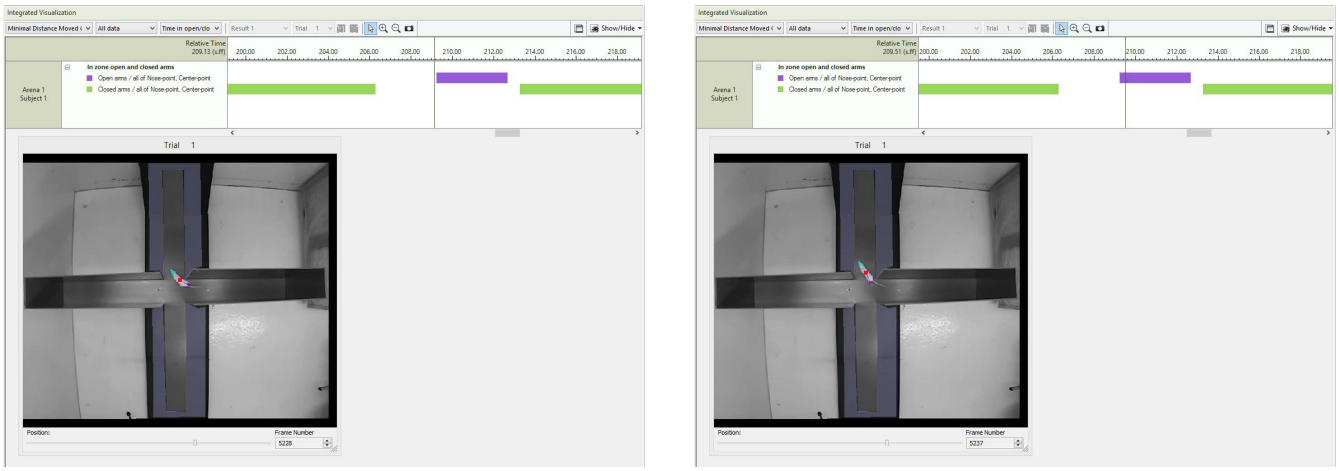
Kocahan *et al.* [1] found that female rats are less anxious than males in the elevated plus maze. They also found a strong correlation between behavioral parameters in a large open field and the elevated plus maze, suggesting these tests investigate similar aspects of anxiety-like behavior.

In the study of Belaya *et al.* [2] results show that voluntary physical exercise stabilized the anxiety level and attenuated the exploration behavior of the 5xFAD transgenic mice in the elevated plus maze. They furthermore demonstrated that this long-term form of exercise reversed cognitive impairment in 5xFAD mice without affecting neurogenesis, neuronal loss, Aβ plaque deposition, or microglia activation. The molecular pathways involved in this modulation could potentially be targeted for benefits against AD.

Afroz *et al.* [3] found that a parental high-salt diet consumption was strongly associated with offspring that shows autism-like behavioral abnormalities via changes in the gut microbiome. In the elevated plus maze, there was a slight tendency toward high-salt diet groups spending less time in the open arms of the elevated plus maze.

Hur *et al.* [4] showed that chronic administration of Methoxphenidine, a novel designer drug, causes addictive and schizophrenia- related behaviours. In the elevated plus maze extreme hyperactivity and increased impulsivity is seen, leading to an increased open arm time compared to controls.

Integrated visualization shows the results of your experiment. In these images, you can see that in this case the animal is registered in the open arm only when both his nose point and center point are detected in the open arm zone, because this was the condition set in the analysis profile



This is seen as abnormal behavior. Methoxphenidine could be used in developing useful animal disease models, but this study also, very importantly, shows that it also requires legal restrictions on its recreational use.

El Boukhari *et al.* [5] are the first to highlight the effects of early thalamic reticular nucleus (TRN) lesions. They provided evidence that early damage of the anterior part of the TRN leads to alterations that may control the development of the thalamocortical–corticothalamic pathways. The elevated plus maze revealed that the TRN lesion group displayed high levels of anxiety.

Albrecht *et al.* [6] showed that PTSD is a complex disorder to behaviorally profile, and thus requires multiple behavioral tests to capture this. They used their behavioral profiling as a tool and identified PTSD-like vs resilient animals. The elevated plus maze was utilized to show that rats with a history of juvenile stress had reduced oped arm exploration, thus increased anxiety-like behavior.

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