Fear conditioning

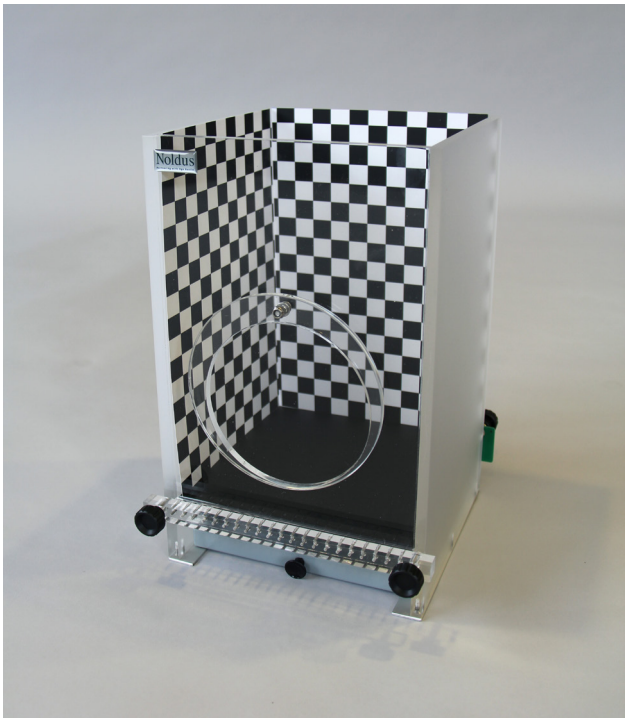
How EthoVision XT benefits your research

A white paper by Dr. Fabrizio Grieco - Noldus Information Technology

FEAR CONDITIONING: A DEEPLY-ROOTED BEHAVIOR

Freezing behavior is usually accepted as a reliable index of fear.

Fear conditioning is a highly-conserved behavior that is found in rodents, in both laboratory and natural situations, and in other animals including humans. It is a form of Pavlovian learning where the subject learns to associate stimuli with a negative (aversive) stimulus or situation. Among the types of fear conditioning, cued and contextual fear conditioning are two separate assays which focus on different neural processes. In cued fear conditioning, a phasic sound or light is associated with an aversive, unconditioned stimulus, usually a foot shock. In context fear conditioning, animals learn the association of the shock with the context, for example, the cage characteristics. Freezing behavior is usually accepted as a reliable index of fear [1-5].

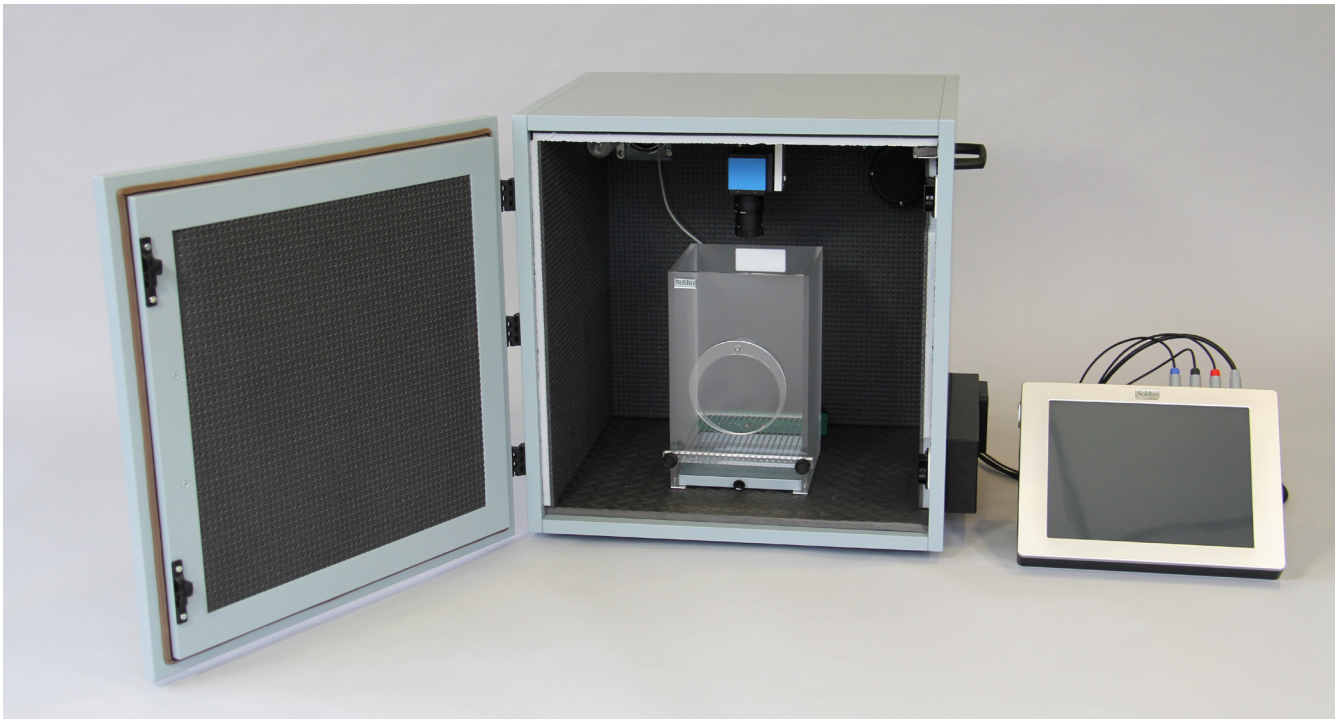


Creating automated tests and reliable detection of freezing behavior are essential for reproducibility of your results. Noldus EthoVision XT combined with the Ugo Basile Fear Conditioning System provides a solution that is centered on just those two aspects.

METHODS

Fear conditioning is a form of one-trial learning. That is, after one conditioning session, subjects show a robust and long-lasting behavioral change.

Testing is done in a sound-proof observational cubicle provided with a camera for video tracking. Infrared lighting and the stimuli: tone, light, and shock. You can control the stimuli directly from EthoVision XT. The protocol is similar between rats and mice; the stimulus intensities used are among the few differences.



EthoVision XT helps you creating routines to administer single or paired stimuli at specific times. You can also specify different protocols for different subjects tested simultaneously.

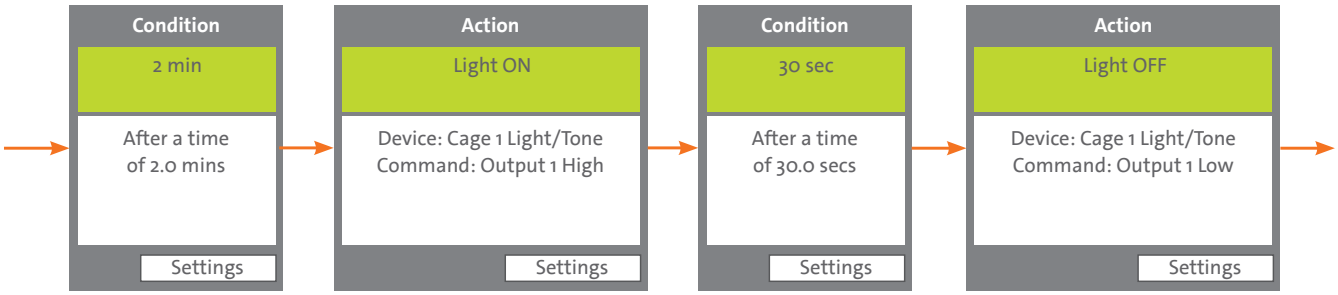
CREATING PROTOCOLS

With fear conditioning, you present the subject with a conditioned stimulus, such as a light, and an aversive unconditioned stimulus, such as a foot shock. EthoVision XT offers an intuitive way to deliver those stimuli for whichever protocol you decide to use.

For example, 2 minutes after the start, turn on the light for 30 sec. This can be represented by a number of conditions (e.g. wait 2 minutes) and actions (e.g., turn the light on and off).

Furthermore, you can combine actions and conditions and have them repeated a number of times in a trial.

EthoVision XT offers an intuitive way to deliver stimuli for whichever protocol you decide to use.



DETECTING FREEZING

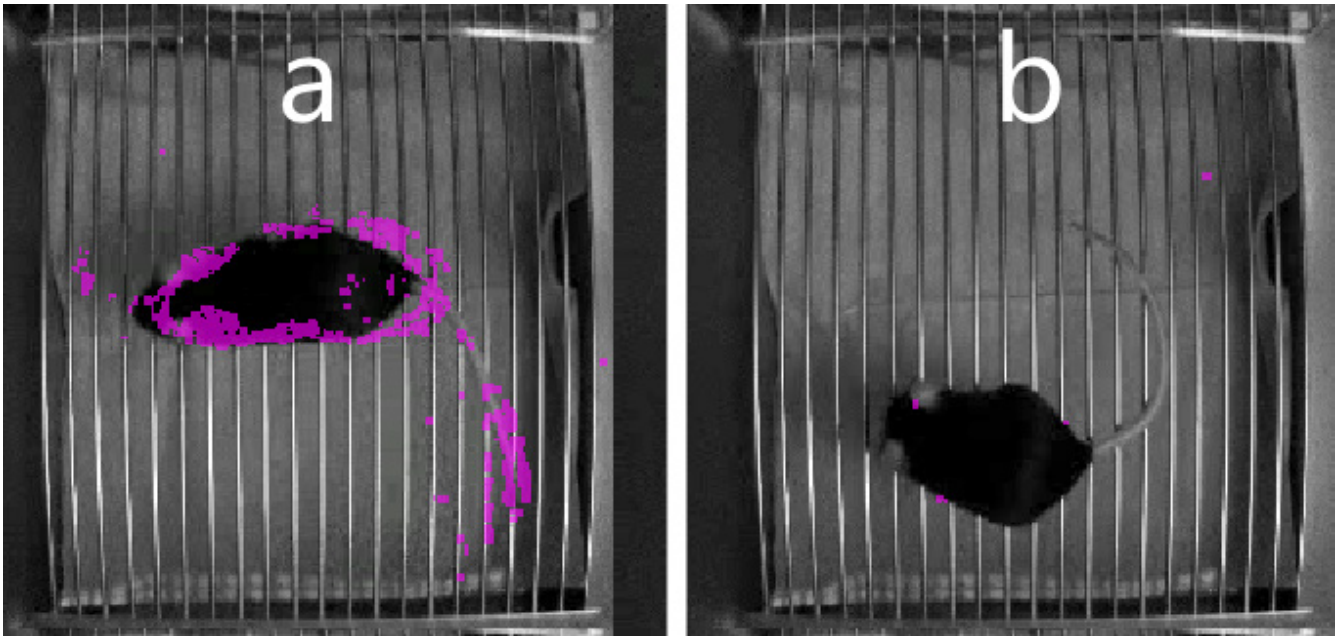
The main parameter is Activity, based on the change in the video image at the pixel level, from frame to frame.

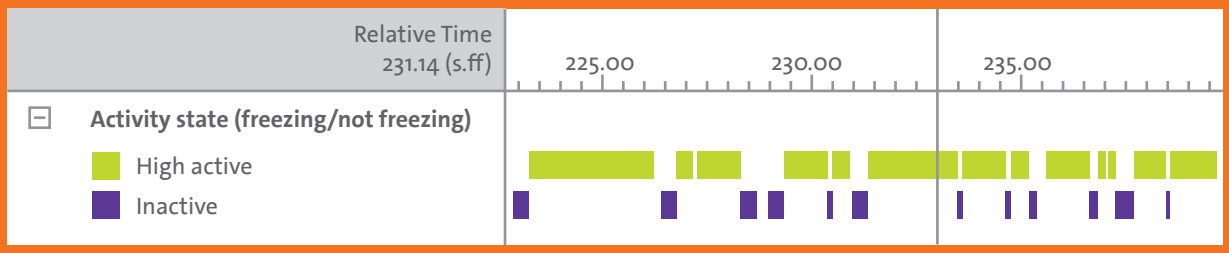
EthoVision XT analyzes the video image using objective and replicable criteria, like the amount of pixel change and the change in the shape of the detected subject. The main parameter is Activity, based on the change in the video image at the pixel level, from frame to frame. This parameter is represented with purple pixels and is high when the subject is active, for example when walking (a), grooming or chewing. When the subject is completely immobile, the amount of image change drops to almost zero (b), making it possible to reliably detect frequency and duration of freezing bouts.

Pham *et al.* [6] used the parameter Mobility to score freezing automatically. According to the authors, EthoVision XT provides parameters that can accurately track mice and be used for automated scoring of immobility that is nearly identical to scoring by human observation.

EthoVision XT is one of the few systems for scoring freezing behavior that has been thoroughly validated [7].

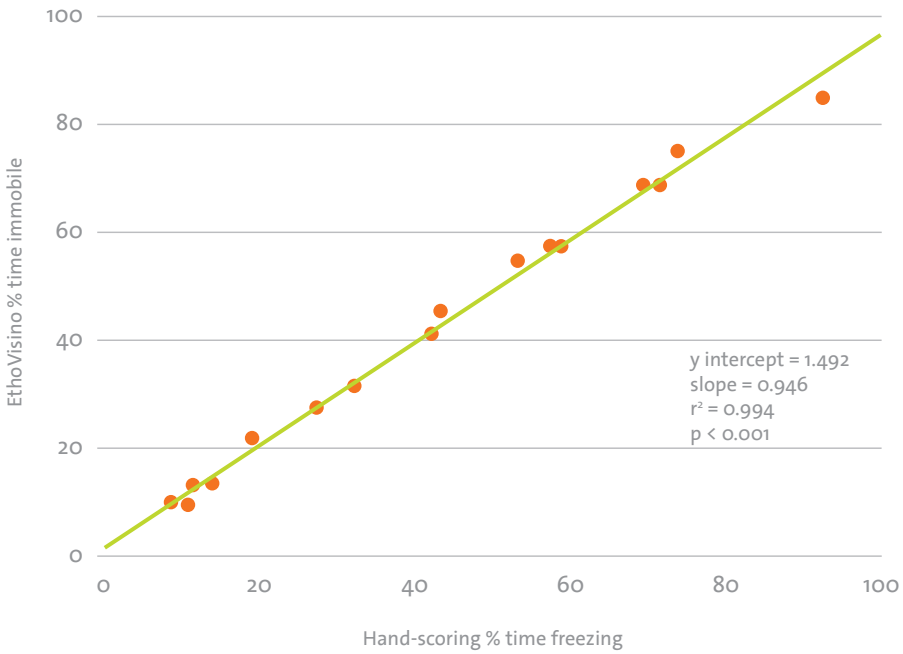
The Activity parameter in EthoVision XT: Purple pixels indicate the subject is active (a) or immobile (b).





The high correlation (r^2 = 0.99) found between EthoVision scores and hand scores of freezing. From Dr. Gina Forster, Automated measures of fear conditioning and extinction in the rat, Satellite Symposium, Society for Neuroscience 2010.

EthoVision XT also offers the capability to score freezing bouts manually. You can then easily compare your scores and the automatic scores on a common timeline, and validate your results.



RESULTS FROM CURRENT RESEARCH

EFFECTS OF GENE OVEREXPRESSION ON FEAR LEARNING

Glutaminase C is an enzyme involved in the process that converts glutamine to glutamate, the most important neurotransmitter in excitatory synapses. A number of neurological diseases have been associated with changes in expression of the Glutaminase C gene. Wang *et al.* [8] generated transgenic mice that overexpressed Glutaminase C in the brain and tested their ability to learn fear. Compared with control mice, transgenic mice Nes-GAC mice had a significantly lower percentage of freezing compared on the last day of Cued fear conditioning, providing evidence that overexpression of Glutaminase C in the brain has deleterious effects on learning.

Freezing was measured using the Activity parameter in EthoVision XT

BLOCKING INTERLEUKIN 1 SIGNALING PREVENTS STRESS-ENHANCED FEAR LEARNING.

There is a general consensus that post-traumatic stress disorder involves substantial immune system dysregulation. Exposure to severe stress is associated with an increase of the signaling molecule Interleukin-1β in the hippocampus. Meghan E. Jones and collaborators (Jones *et al.* [9]) asked whether by simply blocking the interleukin signaling was sufficient to prevent fear learning. The authors used an antagonist to block Interleukin- 1β in the hippocampus. Animals that received severe stress in a specific context followed by the interleukin antagonist infusion showed significantly less freezing than animals that received the same stress followed by vehicle. Freezing was measured using the Activity parameter in EthoVision XT. The authors concluded that infusion of interleukin antagonist prevented the expression of stress-enhanced fear learning.

FEAR CONDITIONING ELICITS RAPID MICROSTRUCTURAL CHANGES IN THE BRAIN

There is substantial knowledge about the anatomical, functional and molecular pathways involved in fear conditioning. Establishment of persistent long-term fear memory requires structural changes in the synapses in specific regions of the brain, particularly the amygdala and hippocampal projections. Abby Ding and collaborators (Ding *et al.* [10]) used in vivo magnetic

resonance diffusor tension imaging (DTI) before and after fear conditioning. To record freezing behavior in the subjects, the authors used the EthoVision XT parameter Mobility which analyzes subtle changes in the contour of the animal viewed from the camera in the fear conditioning chamber. The DTI revealed rapid microstructural changes in the amygdala and hippocampus in vivo, as early as one hour after conditioning. The study confirmed that the two areas of the brain are the most important in the regulation of fear.

Depletion of Rheb GTPase signaling molecule elicits memory defects in mice

Ras genes encode for signaling molecules that usually work as two-state switches. If activated, a Ras molecule triggers other molecules, and ultimately determine signal pathways within the cell. Rheb (Ras homolog enriched in brain) GTPase is one of such molecules; it interacts with cell targets that are known to be implicated in life-span extension, as well as in neurodegenerative diseases such as Alzheimer's disease. However, it is not clear how Rheb GTPase participates in aging and neurodegenerative disorders. Shahani and collaborators (Shahani *et al.* [11]) crossed mice with Rheb with transgenic mice expressing an inducible promoter which depletes Rheb molecules. The mutant mice were then subject to contextual fear conditioning and the occurrence of freezing was recorded. Mice showed the same amount of freezing as controls. However, they performed less in spatial memory paradigms compared to controls, suggesting that Rheb depletion elicited spatial memory deficits.

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