# Video-Based Analysis of Fear Conditioning: A Validation Test

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## Introduction

Fear conditioning is a form of Pavovlian learning, where an electric shock is paired with an acoustic stimulus. Animals learn the association of the aversive stimulus (shock) with the auditory one, but also with the context where the shock takes place [1].

This type of learning is classified as a form of non-declarative memory and has several advantages. First and foremost is a form of one-trial learning, which is acquired rapidly and requires only one conditioning session, after which a robust and long-lasting behavioural change is produced. Moreover, it doesn't require food or water deprivation. Finally, fear conditioning is an important basic learning mechanism, which is common both to human and non-human animals and its neural mechanism appear to be relatively conserved across species [1-3].

The main behavioural measure in fear conditioning is the number and duration of freezing episodes, which may be scored by a trained observer or toward automated scoring system [1]. We would like to validate automatic scoring using the EthoVision® 8.5 software (Noldus Information Technology, Wageningen, The Netherlands) in both mice and rats, and further analysed by The Observer® XT (Noldus Information Technology).

## Materials and Methods

Fear conditioning is carried out in both mice and rats, using the new Fear Condition System by Ugo Basile (Ugo Basile, Comerio, Italy). The system comprises a shock chamber, made of three/four black/white acrylic walls, the ceiling of white acrylic, the floor of the chamber is made of metal bars that deliver the electric foot shock. It also consists of a shock generator for the administration of shocks at various intensities, a tone generator, and an isolation cubicle.

We will test fear conditioning in two different protocols; in particular, the experiment consists of two sessions, a training session, and a test session. On day 1 the training is performed, the animal is placed in the conditioning chamber. The animal is left free to explore the chamber for

- Protocol A) 2 min before the presentation of the acoustic stimulus (30 s) followed by the presentation of the foot shock (2 s). [1]

- Protocol B) 1 minute before the presentation of two tone-shock pairings with an acoustic stimulus (20 s) and a 500 ms long electric shock [4]

The second day the test is performed.

During context test the subject is placed in the same conditioning chamber that is used for training. However, no shock and no tune cues were delivered.

During the cue test the animal is placed in a modified chamber, i.e. without the metal bars on the floor, which is replaced a normal floor, and with modified walls. The tone cue/s is/were presented as in the training session.

Rodent behaviour is recorded by a video camera and automated tracking is performed using EthoVision® XT 8.5 software (Noldus Information Technology, Wageningen, The Netherlands). The following behavioural elements were recorded: total freezing time, number and duration of freezing episodes, locomotor behaviour, escaping to one corner of the cage [5].

## Ethical statement

The experiments received authorisation from the Italian Ministry of Health, and are conducted following the principles of the NIH Guide for the Use and Care of Laboratory Animals, and the European Community Council (86/609/ EEC) directive. Mice are housed and used according to current European Community rules. Experiments on mice are approved by the research committees from the University of Verona and Italian National Institute of Health.

## References

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