Practical analysis of behaviour

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22/05/2023

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Standard Behavioural tests

Non-invasive methods to study behaviour and the brain regions associated by choosing different tasks related with:

- Social behaviour
- Anxiety/ stress
- Motor coordination e motor ability
- Learning and memory

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Social tests

Three-chamber sociability

https://med.stanford.edu/sbfnl/services/bm/si.html

Habituation: Empty Apparatus

Sociability: Novel Object; Mouse 1

Social Novelty: Mouse 1; Mouse 2
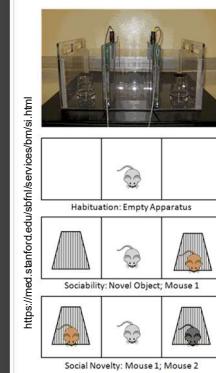
Mouse-to mouse contact behaviours

Nose   Body   Anus

https://www.kobeu.ac.jp/research_at_kobe_en/NEWS/news/2020_10_01_01.html

Dominance tube test

Release Site   Release Site

Animals interact in center

Winner   Loser
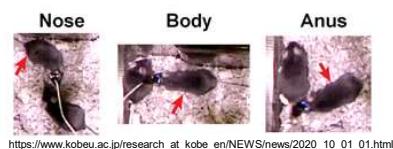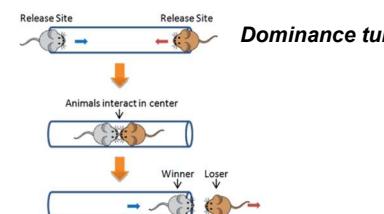
Social tests
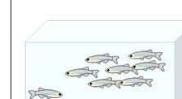
Shoaling test   **Social preference test**
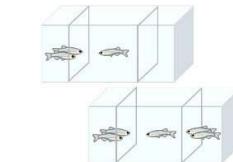
Social interaction test   **Mirror test**
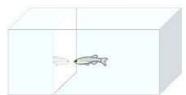
https://www.mdpi.com/1422-0067/20/6/1296

Standard Behavioural tests

- Social tests

- Anxiety/ stress tests

- Motor coordination e motor ability tests

- Learning and memory tests

Stress/ anxiety tests

- **Active/passive avoidance**
- **Elevated plus-maze**
- **Open Field**
- **Light/Dark box or White/ black box**
- **Novel tank test** (zebrafish)

- Based on the tendency of vertebrates to avoid a site that is innately aversive or that has become aversive through conditioning

- Automatic test and analysis (time spent in different sites of the apparatus)

Elevated Plus Maze

Conflict between motivation to explore and aversion to unprotected places


Automatic analysis
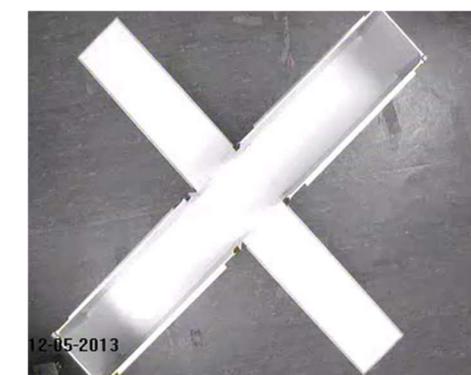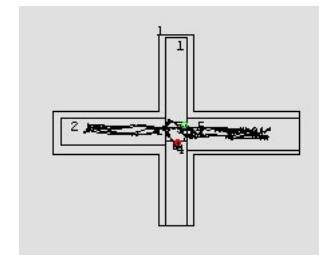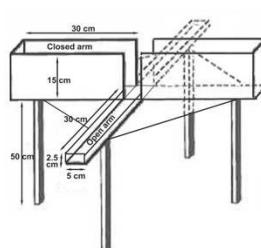
Anxiety tests

Novel tank
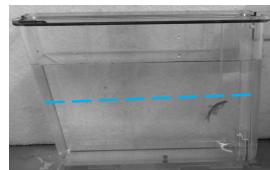
White/ black box
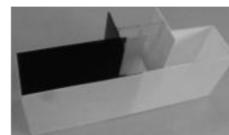
Swimming plus maze test
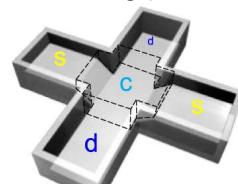
Thigmotaxis analysis

Swimming Plus-Maze test

deep arms

centrer zone

shallow arms

% time

% time
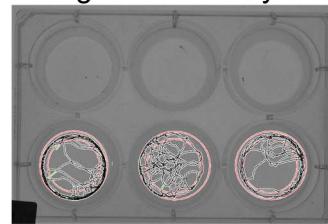
distance from center point
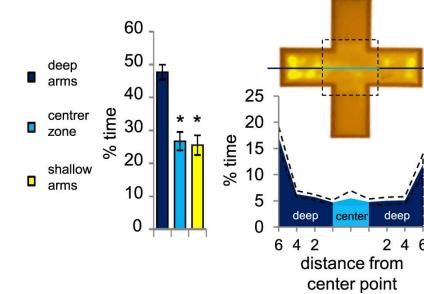
Varga, Z. K. et al. (2018). *Scientific reports*, *8*(1), 16590
(for juvenile zebrafish and larvae)

Novel tank 3D tracking

A   Temporal

B   Spatial

Time, s

Velocity, m/s
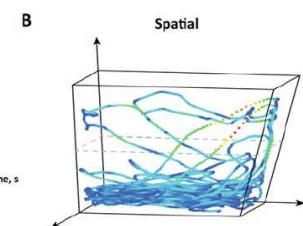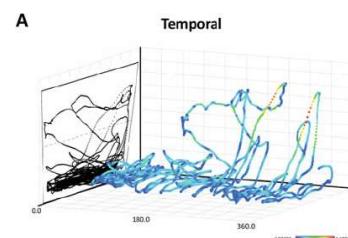
Cachat, JM et al. (2011). In *Zebrafish neurobehavioral protocols* (pp. 191-201). Humana Press.

Standard Behavioural tests

- Social tests

- Anxiety/ stress tests

- Motor coordination e motor ability tests

- Learning and memory tests

Rotarod test

- Automatic equipment
- Latency to animal to fall
- Motor learning or motor coordination and balance

Precision in the movements, speed
and coordination is important to
survive in the natural habitat.

(Other tests: treadmill, pole test,
ladder test.)

Rotarod test

Ladder test

Swimmill

- Swimming capacity of zebrafish
- Ucrit : the highest water velocity when zebrafish continued to swim for whole 1 min.
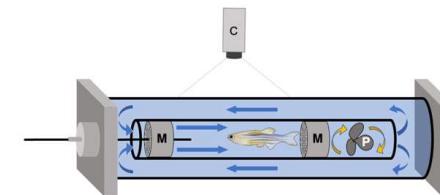
Wakamatsu, Y et al. (2019). *Scientific reports*, *9*(1), 1-8

Standard Behavioural tests

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Mazes – learning and memory

Orientation to find the reward, the shelter or the safe location form an aversive situation

➢ Cues inside the maze (visual, olfactory, auditory, touch)

➢ To memorize the body movement

Non-spatial strategy

➢ Cues outside the maze (visual)

Spatial strategy

Mazes

Motivation to explore new places and find a reward

T-maze
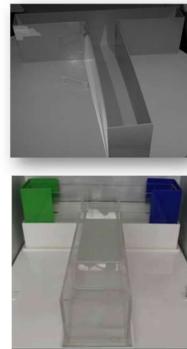
Y-maze

Radial arm maze
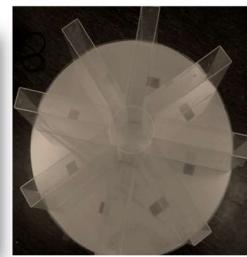
Mazes

Motivation to escape from an aversive situation finding the exit/ safe place

Water maze

Barnes maze

Conditioned place preference or aversion

http://www.youtube.com/watch?v=WjwA8c0Xh7Y

One-trial inhibitory avoidance

Object location/recognition

Based on the innate ability of exploration and the preference for novelty

Sample phase

interval → Choice phase

Object recognition

interval →

Choice phase

Object location

Tracking

Automation may not be sufficient
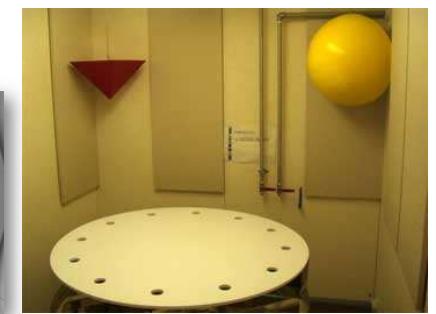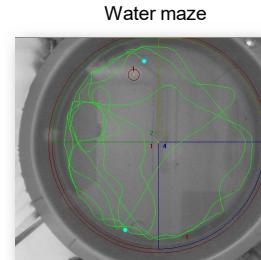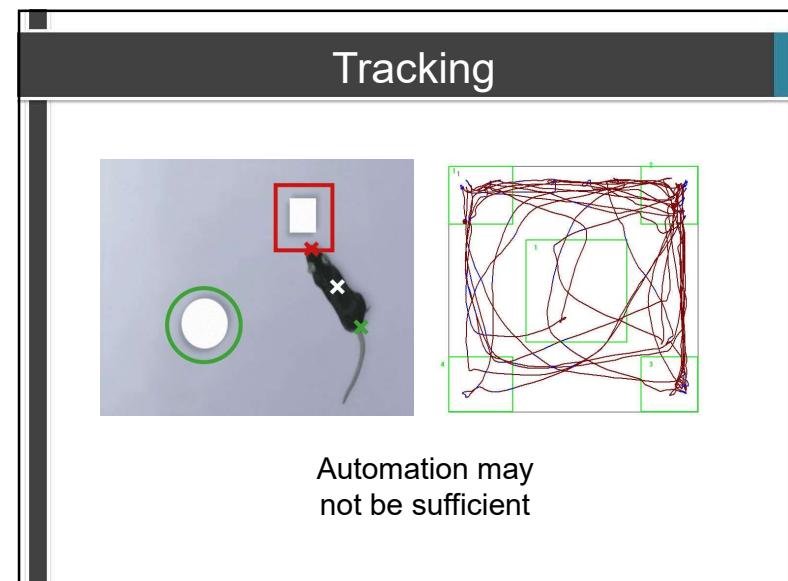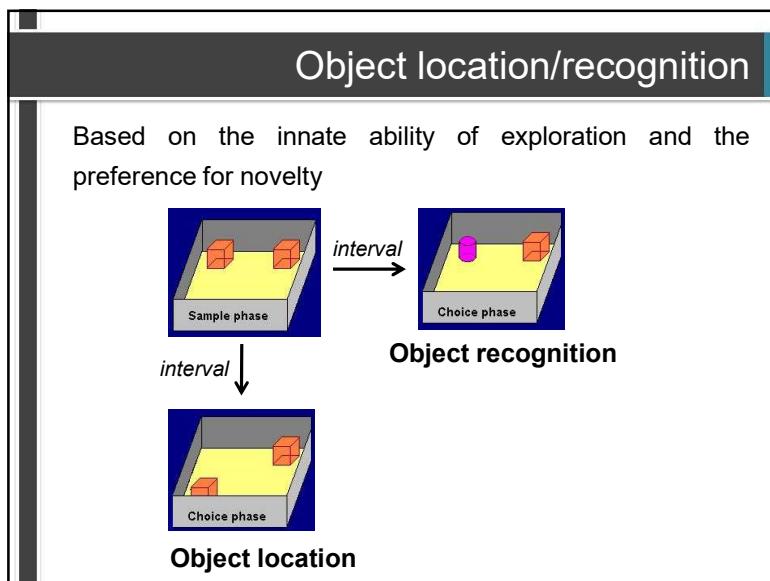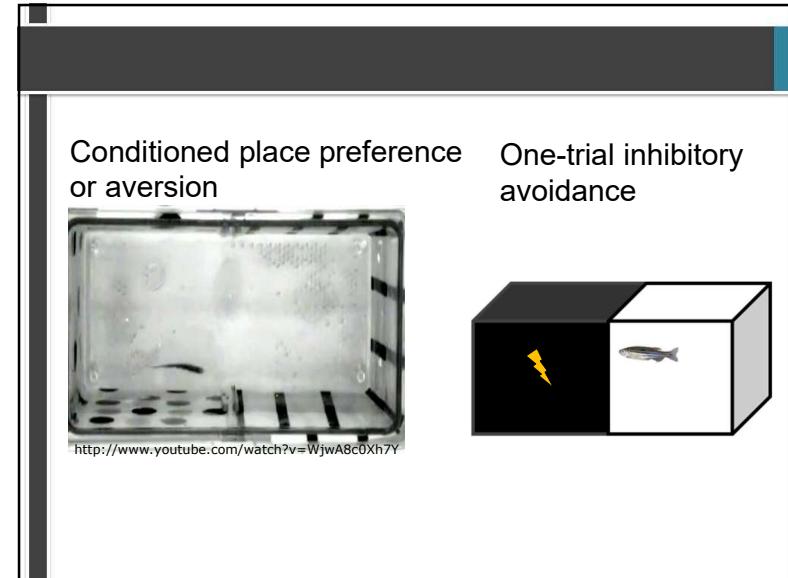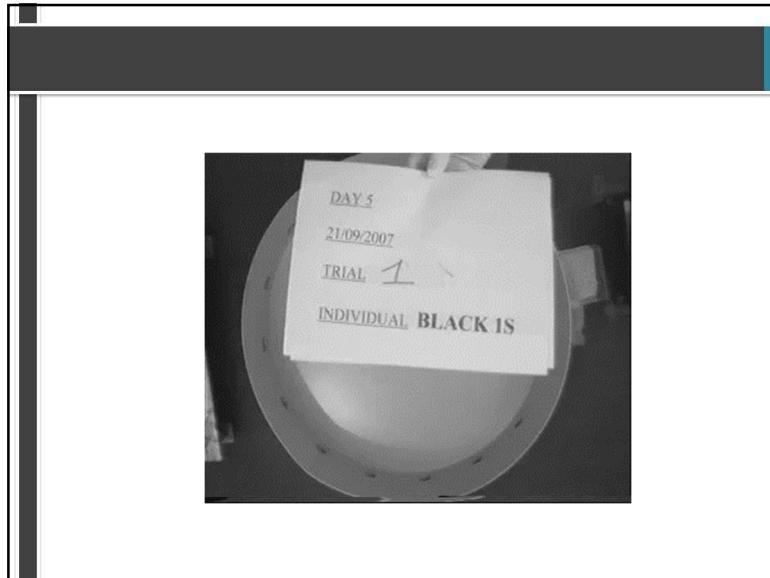
Ethological validity

- Detailed ethological analysis:

Rearing

Head dip

Strech attend posture

Defensive burying

Grooming

Augustsson 2004 http://diss-epsilon.slu.se/archive/00000531/
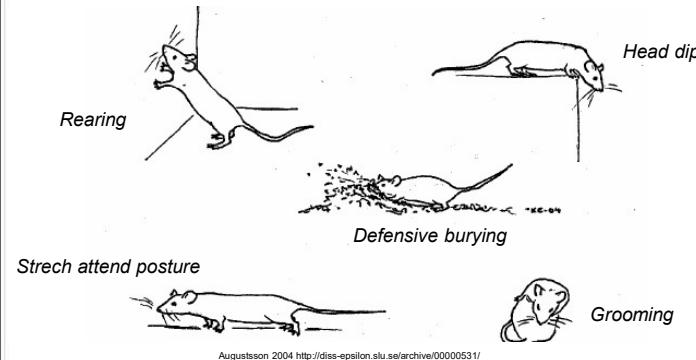
Test validation

- To validate the material and the protocol.
- Pilot tests.
- Animals without treatment must have expected behaviours to compare (literature, natural behaviour)
- ex.: Anxiety tests: use of anxiolytics

     Learning tests: use of environmental enrichment

        use of cognitive enhancers or inhibitors

Environmental enrichment

Rats from ENRICHED environment present
- Bigger dendritic fields
- More synapse per neuron
- Better performance in memory tasks

(Benefiel & Greenough 1998)
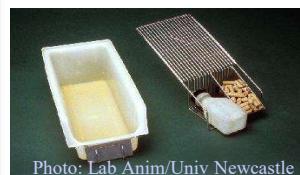
Photo: Lab Anim/Univ Newcastle

Why consider ethology

- To avoid results without validity if the animals cannot adapt to the environment, stimuli used or housing
- To get tests more sensitive
- To get information from natural behaviours
- To develop new valid tests

Experimental design

- **Field** or **laboratory**

- **Descriptive** or **experimental**

The choice depends on
- Research question
- Specie
- Available information of previous studies
- …

Methods for data collection

Sampling

- *Focal animal sampling* – one individual is observed by a specific period and all its behaviours registered

- *Scan sampling* – one groups of individuals is scanned regularly and the behaviours of all animals are registered.

Methods for data collection

Registration

- *Continuous sampling* – all the behaviours are registered from the beginning to the end

- *Instantaneous sampling* – the behaviour is registered at defined intervals

Methods for data collection

- **Direct observation**
- **Video recording**

- To talk to a recorder
- Registration/ observation sheets
- Computer with specialized software
(Automated tracking software or event-coding software)

Ethogram

- List of behaviours to observe and register
- All the behaviours possible or pre-defined behaviours related with the research question
- Detailed and objective descriptions of the behaviours
- Simple registration codes
- To make the apparently subjective in objective observations

To establish an ethogram

Object recognition test

- Mice strain: C57Bl/6J
- Different treatments
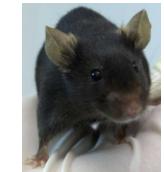
M&M

- Recognition of the familiar object
- Previously, habituation to the apparatus

Sample trial   **Choice trial**

Interval →

03-2014   03-2014

Exercise

7/3/2014 ID 52

07-03-2014

Exemplo:

- **Aproximação ao objecto**: o animal movimenta-se em direcção ao objecto e o comportamento é registado quando o seu nariz entra de 5 a 2 cm à volta do objecto.
- **Explorar o objecto:** o animal toca com o nariz no objecto ou o nariz está direcionado para o objecto a uma distância inferior a 2 cm. Estar de costas para o objecto, circunda-lo ou ir para cima dele não é considerado explorar o objecto.
- **Freezing**: animal encontra-se totalmente imobilizado numa postura tensa/ rígida, sem movimento das vibrissas. Por vezes este comportamento inicia-se com uma paragem brusca do animal.

Elaboração etograma

Exemplo:

**Postura de avaliação do risco:** o animal estica o seu corpo, avançando apenas as patas dianteiras. O seu ventre fica perto do chão e o animal cheira o chão ou o ar. O animal pode deslocar as patas dianteiras para os lados, permanecendo com o corpo esticado. O comportamento acaba quando ele volta com as patas à posição inicial e/ou quando o corpo já não está esticado.

- **Grooming:** animal encontra-se parado, lambe as patas e limpa o nariz, a cara movimentos rotatórios de trás para a frente que podem chegar até atrás das orelhas. Pode ainda fazer a limpeza do corpo limpando com a pata e lambendo as partes laterais do seu corpo ou virar-se para limpar a cauda da mesma forma.
- **Rearing:** animal encontra-se apoiado nas suas patas traseiras numa posição vertical (bípede), com a coluna estendida. As patas da frente podem estar junto sem qualquer apoio ou podem-se apoiar numa superfície vertical firme (parede). Normalmente a cabeça está levantada e esticada para cima.
- **Outros** (O) Quaisquer outros comportamentos não abrangidos pelas anteriores categorias


Parameters/ measurements

- Frequency

- Duration

- Latency

"Event logging"- Observer

http://www.noldus.com/animal-behavior-research/products/the-observer-xt

"Event logging"- Observer

"Event logging" - Boris

Event logging - Ethowatcher
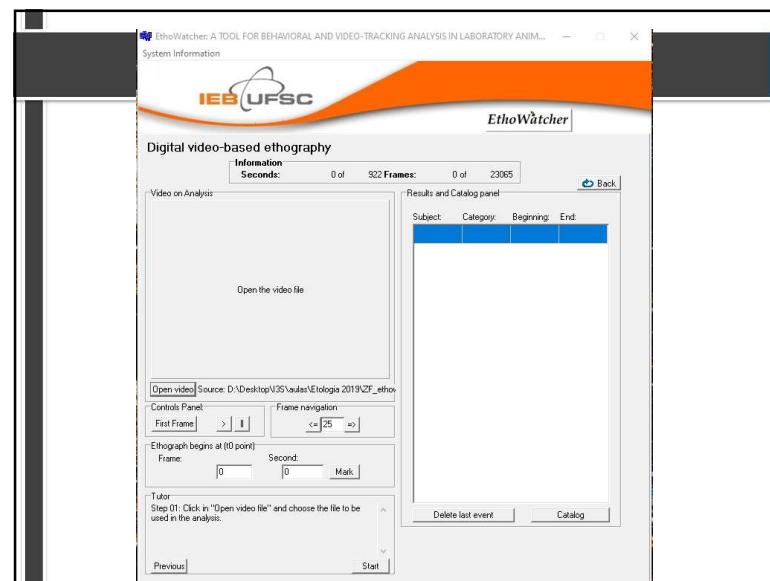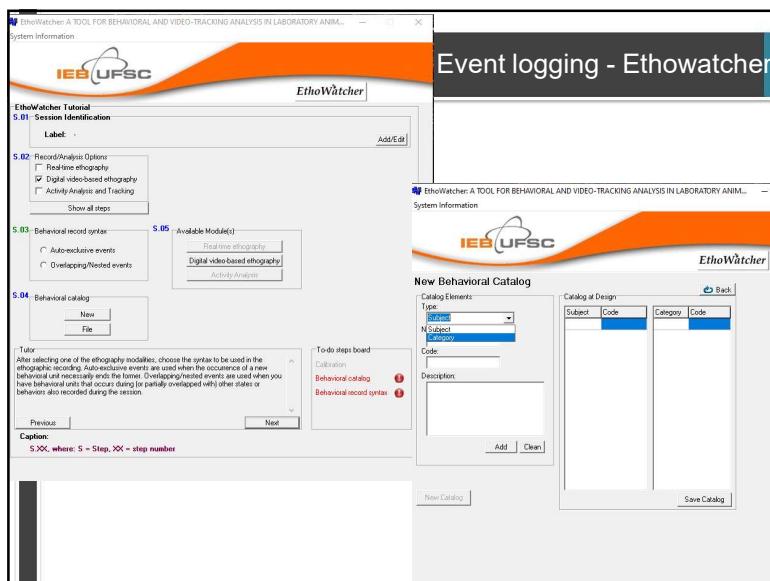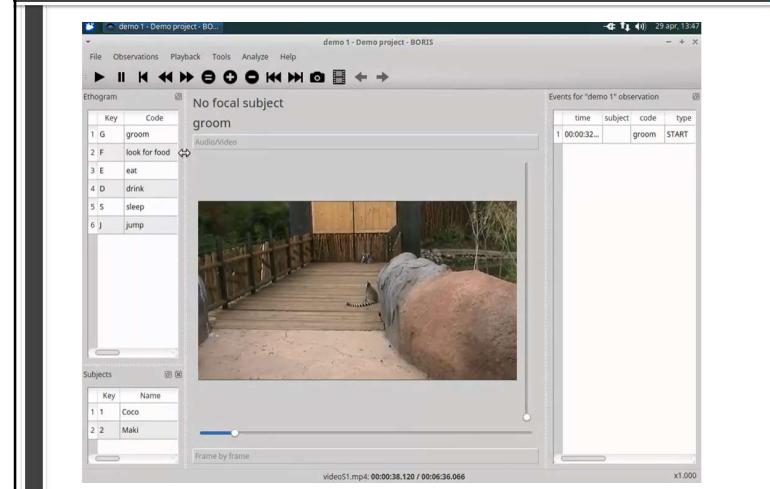
Automatization on behaviour recognition?

http://www.noldus.com/EthoVision-XT/Rat-Behavior-Recognition
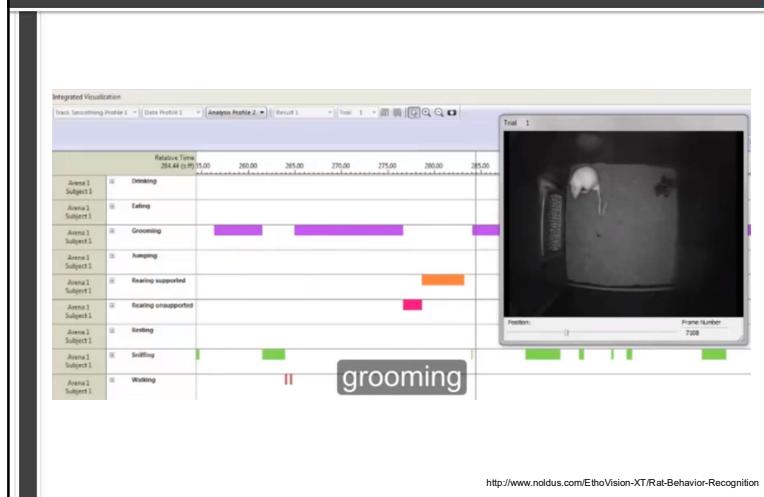
Tracking- TheRealFishTracker
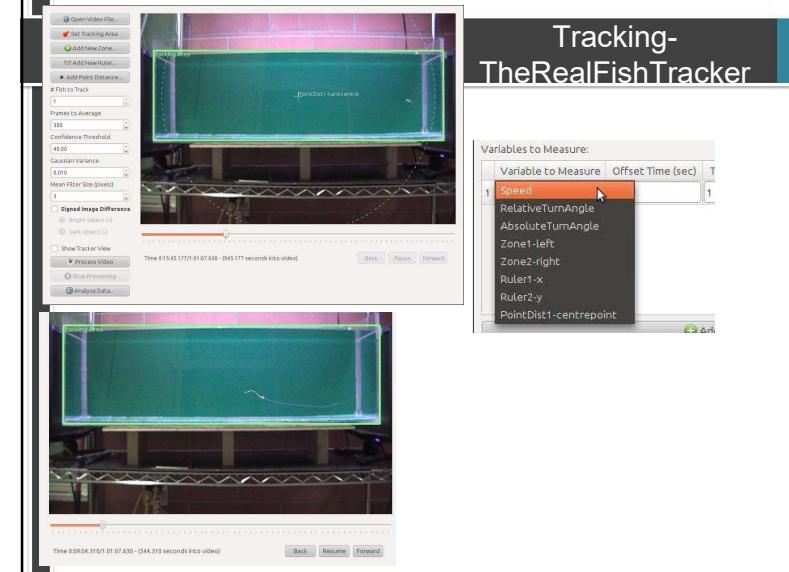
Software for behavioural analysis

- Event Logging
- Observer (Noldus)
- VideoMot2 (TSE systems)
- EthoWatcher (free)
- BORIS (free)
- Etc…

- Tracking software
- Ethovision (Noldus)
- VideoMot2 (TSE systems)
- Anymaze
- TheRealFishTracker (free)

Subjective became objective...

- Objective and detailed description of the behaviour, allowing repeated observations with different observers

- When possible, observer must be **blind** to the treatment/ group

Randomization

- Animals per treatment
- Object recognition: objects role and position of the new object within groups

Internal validation

Improved the validity of the results about 44% (35-53%)

Macleod et al, Stroke. 2008; 39: 2824-2829