

Automated behavioral phenotyping of mice

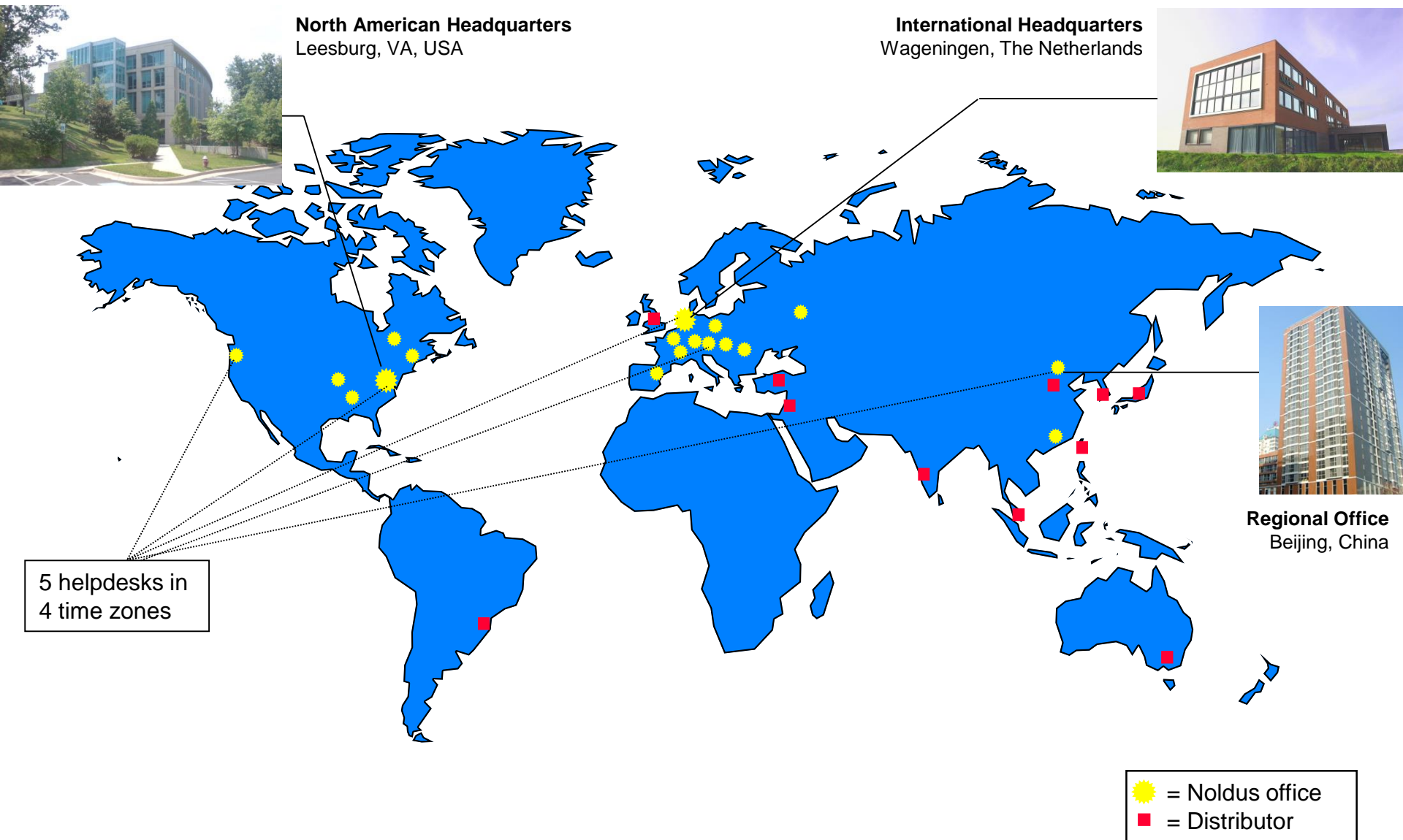


Lucas P.J.J. Noldus, Ph.D.

INFRAFRONTIER / IMPC Industry Liaison Meeting
Barcelona, Spain, 13 November 2014

- Developer of software, hardware and integrated solutions for behavior research
- Founded in November 1989 by Lucas Noldus
- 130 employees worldwide
- Headquarters in Wageningen, The Netherlands
- Offices in France, Germany, Spain, Hungary, USA, Canada, China
- ~ 7,000 customers in 85 countries





Conventional behavioral phenotyping approach

- Animal is taken from home cage and placed in test apparatus
- Test lasts short amount of time (e.g. 5–30 minutes)
- Test takes place in light or dark period
- After test, animal is returned to home cage
- Each test focuses on one aspect of behavior
- Screening one mutant mouse requires series of different tests



Open field



Object recognition



Morris maze



Light-dark box

Drawbacks:

- Designs and procedures not standardized
- Test addresses only one motivational system
- Test renders one or few end-points
- Short observation period



Elevated plus maze

Drawbacks of conventional methods

- Series of different tests: very **time-consuming** and expensive
- Tests require **human operator** (animal handling, test supervision): severely limits throughput
- Use of test batteries implies **short observations** at random moments of the day/night cycle: important behavioral or physiological events may be missed
- Initial response to **novelty** may obscure behavioral differences
- Frequent handling and transport from/to home cage induces high **stress** levels in the animals
- Tests apparatus and protocols are **not standardized**: many (hidden) confounding factors

- Rather than taking the animal to the test apparatus, bring the test to the animal's home cage!
- Replace battery of 1-dimensional tests by one multi-dimensional test protocol
- Investigate animals in a more natural environment → normal performance can optimally flourish and dysfunctions become apparent
- Replace human operator by fully automated system
- Test in dark period: observe rodents when *they* are awake

Phase 1

- Project: Neuro-Bsik Mouse Phenomics
- Duration: 2003-2009
- Budget: € 26 million (50% subsidy)



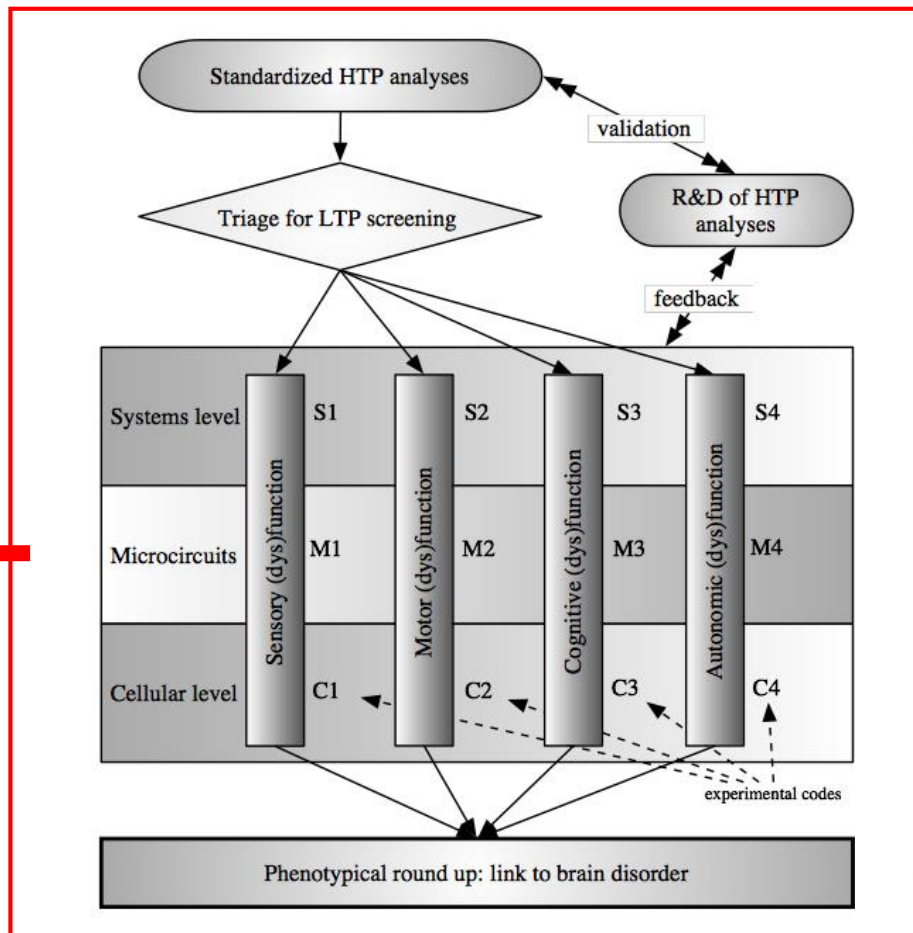
Phase 2

- Project: NeuroBasic PharmaPhenomics
- Duration: 2010-2015
- Budget: € 25 million (50% subsidy)



Focus on mouse models of neurological and psychiatric diseases





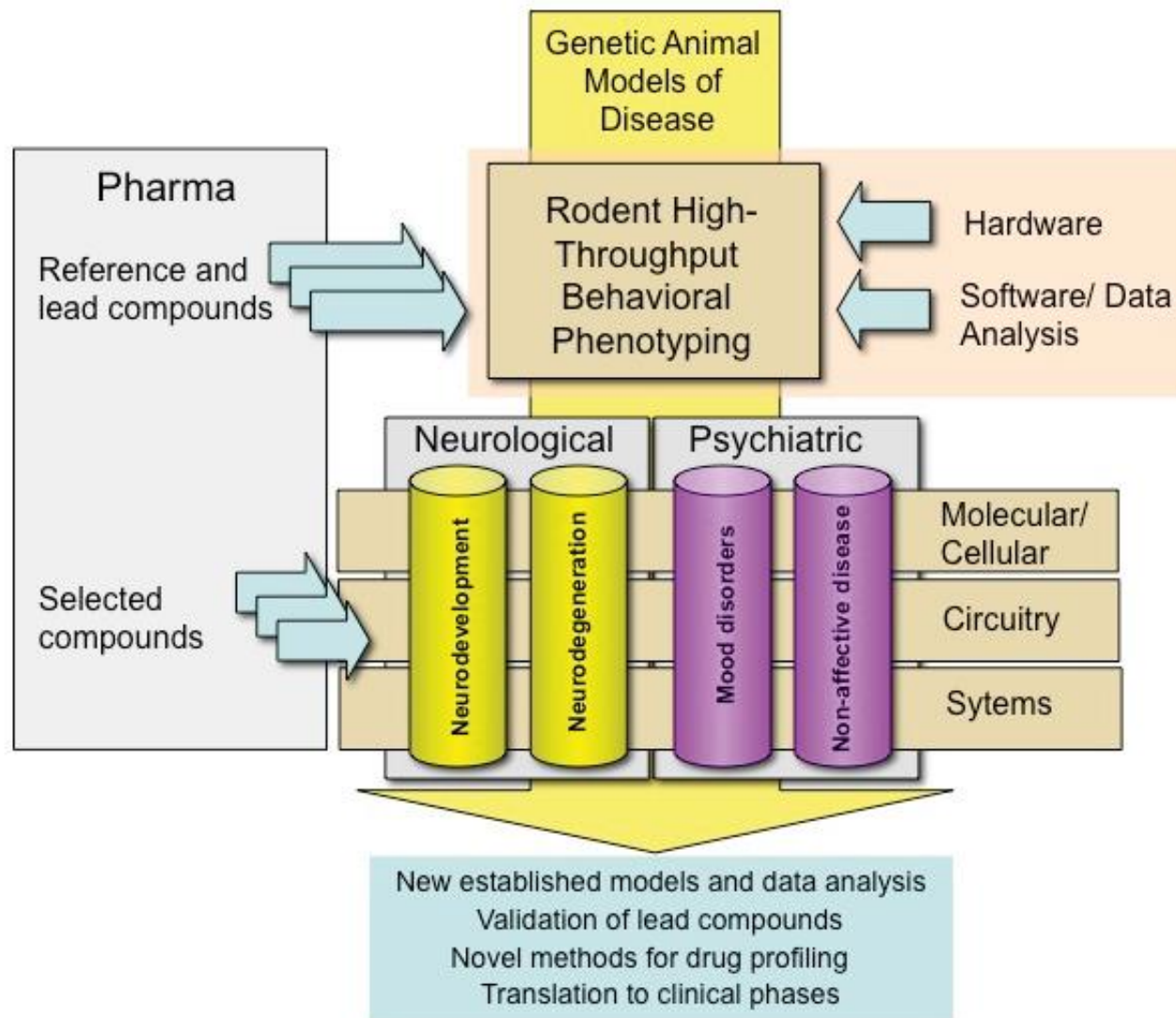
Knowledge:
- Technology
- Science

HTP products:

- Hardware
- Software
- Services

Mouse models:

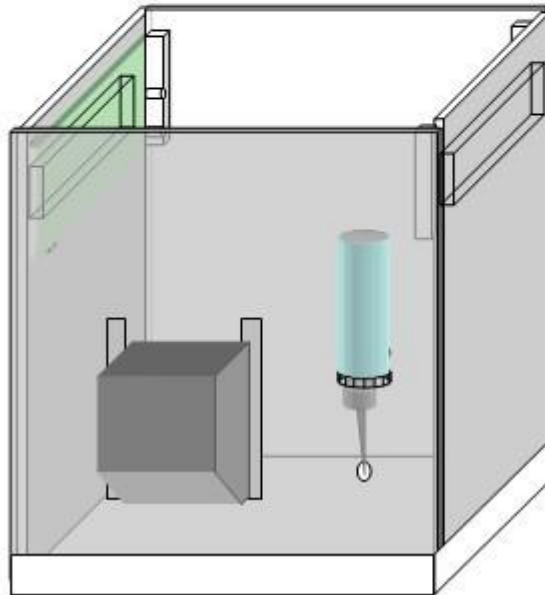
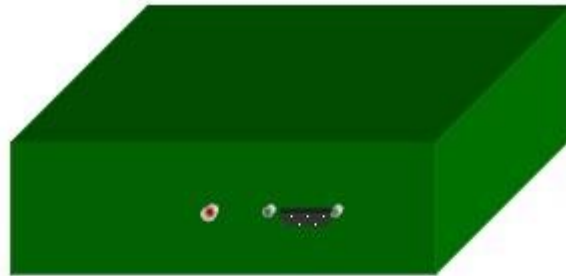
- Pharmaceutical industry
- Research institutes



Automated behavioral testing in home cage

- Zero human interference: no animal handling or transportation
- 24/7 continuous measurement: more data for less effort, nothing is missed
- Flexibility: sensors and stimuli can easily be added or removed
- Rich and varied environment (food, drink, shelter, bedding): maximizes animal welfare and behavioral opportunities during test





Top unit

- Video camera
- IR LEDs
- Buzzer
- White spot light
- Yellow cue light

Replaceable walls

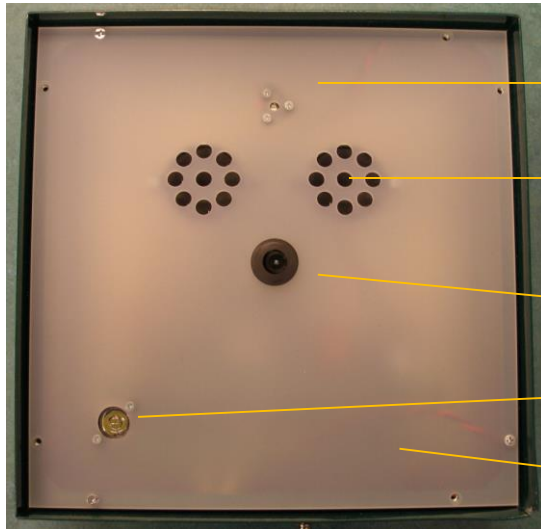
- Drink bottles
- Feeders
- Running wheel
- Operant devices

Choice of shelters

- White
- Black
- IR-translucent
- With illumination

Choice of bottom plates

- White
- Black



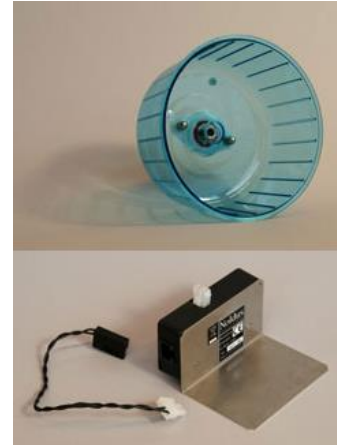
Yellow cue light

Ultrasound generator
(optional)

Video camera

White spot light

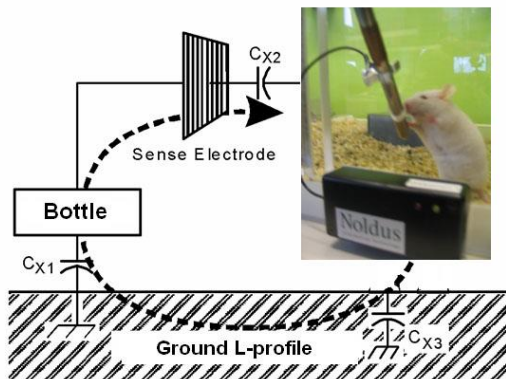
Buzzer



Running wheel



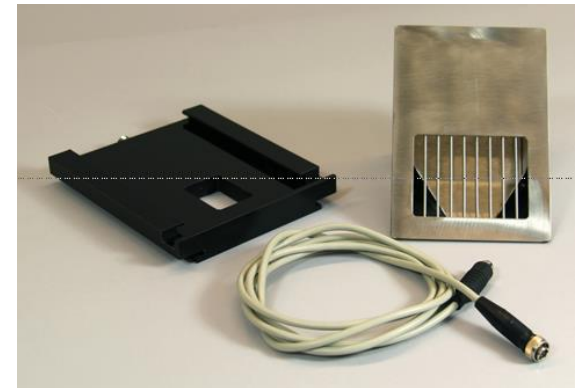
Pellet dispenser



Lick-o-meter



Illuminated shelter



Feeding monitor

Nose-poke Sensor - 8 mm illuminated LED



Nose-poke Sensor - 8 mm Hole for pellet dispenser



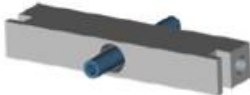
Nose-poke Sensor - Positive reward (water)



Liquid-reward with Lick-sensor (resistive)



Liquid-reward with Lick-sensor (piezo mechanic)



Motorised flap



Negative Reward (air puff with valve)



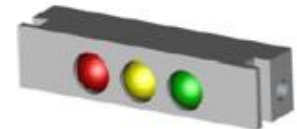
Odour Port



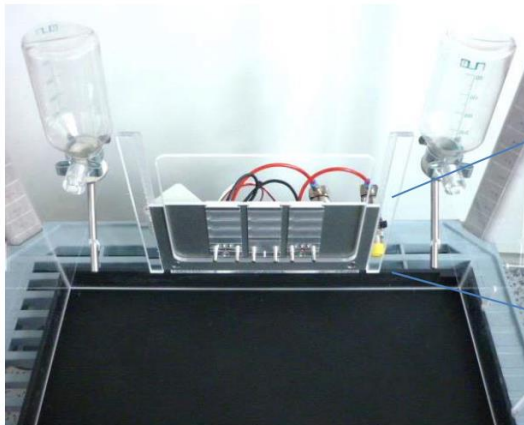
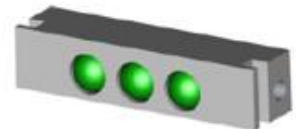
Odour Port with Nose-poke Sensor

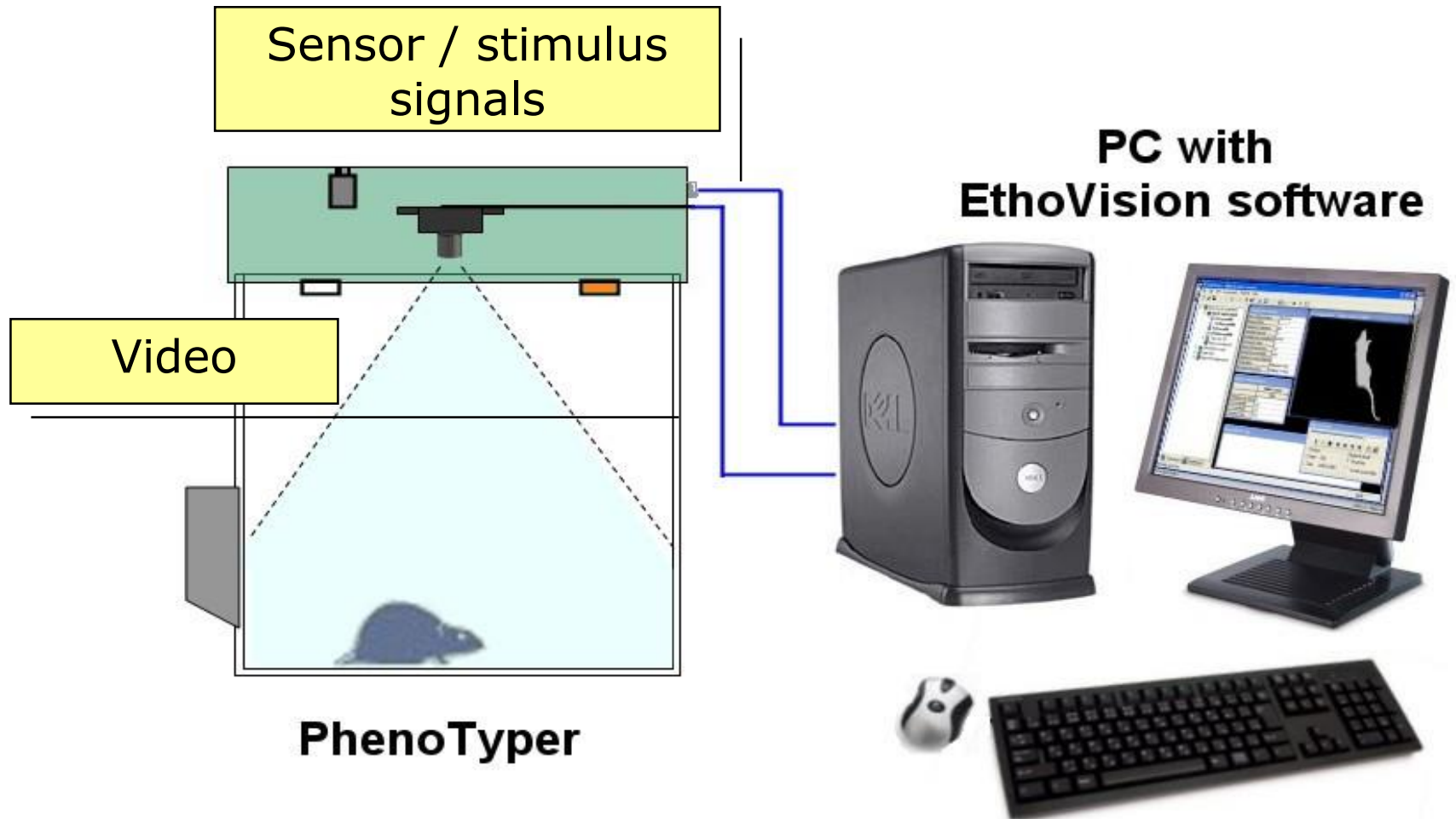


3-colour Triple Light



Triple Light (green)





EthoVision XT - Demo1

File Edit View Setup Acquisition Select Visualize Analyze Export Help

Type a question for help

Experiment Explorer

- Demo1
 - Experiment Settings
 - Trial List
 - Arena Settings (1)
 - Trial Control Settings (2)
 - Trial Control Settings 1**
 - Trial Control Settings 2
 - Detection Settings (1)
 - Track Smoothing Profiles (1)
 - Data Profiles (1)
 - Analysis Profiles (1)
 - Acquired Trials (0)

View Settings

Components	Show	Set
Components	<input checked="" type="checkbox"/>	
Maximum trial du...	<input checked="" type="checkbox"/>	

Components

Structures

- Sub-rule
- Sub-rule reference
- Operator (any, all, ...)

Conditions

- Time
- Time interval
- Trial Control variable

Dependent variables

- Velocity
- Movement
- Distance moved
- In zone
- Mobility
- Distance to zone
- Heading
- Turn angle
- Angular velocity
- Distance to point
- Rotation
- Zone transition
- Meander
- Heading to point

Hardware

- Top Unit (Standard)
- Pellet Dispenser (PTPD-0010)
- Lickometer (PTLM-0010)
- Custom Hardware

Actions

- Trial Control variable

Hardware

- Top Unit (Standard)
- Pellet Dispenser (PTPD-0010)
- Custom Hardware

Maximum trial duration

The duration of tracks can never exceed the maximum trial duration.

☐ Use a maximum trial duration

of 10 mins

Rule Begin

Start-stop trial

Condition

In zone (1)

Duration ≥ 1 s

Action

Start track

Reference

Feeding Reference

To: Feeder Zone

Start: without d...

Repeat indefinitely

Stop to Start 00:...

Settings...

Reference

Drinking Reference

To: Drinking

Start: without d...

Repeat indefinitely

Stop to Start 00:...

Settings...

Operator

Operator (1)

Any input True

Settings...

Condition

Time (1)

Undefined delay (condition never met)

Settings...

Action

Stop track

Rule End

Start-stop trial

Rule Begin

Feeder Zone

Settings...

Condition

In feeder zone

Current = true

Settings...

Action

Drop food pellet

Device: Device C

Command: Drop pellet

Settings...

Condition

Out of Feeder Z...

Current = true

Settings...

Rule End

Feeder Zone

Rule Begin

Drinking

Settings...

Condition

If Drinking

Device: Device B

Signal: Number ...

Value: ≥ 1

Settings...

Action

Light on

Device: Device A

Command: Whit...

Settings...

Condition

Time Delay

After a delay of 1.0 secs

Settings...

Action

Light off

Device: Device A

Command: Whit...

Settings...

Operator

Operator (2)

All inputs True simultaneously

Settings...

Rule End

Drinking

Action

Sound on

Device: Device A

Command: Soun...

Settings...

Condition

Time Delay2

After a delay of 0.1 secs

Settings...

Action

Sound off

Device: Device A

Command: Soun...

Settings...

Quantitative parameters (selected list)

Distance and time

- Time in zone
- Distance to zone
- Distance to point
- Distance moved
- Velocity

Path shape

- Heading
- Turn angle
- Angular velocity
- Meander

Individual behaviors

- Moving / not moving
- Mobile / highly mobile / immobile
- Body elongation
- Body orientation
- Head direction
- Rotation
- Grooming, sniffing, twitching (*)

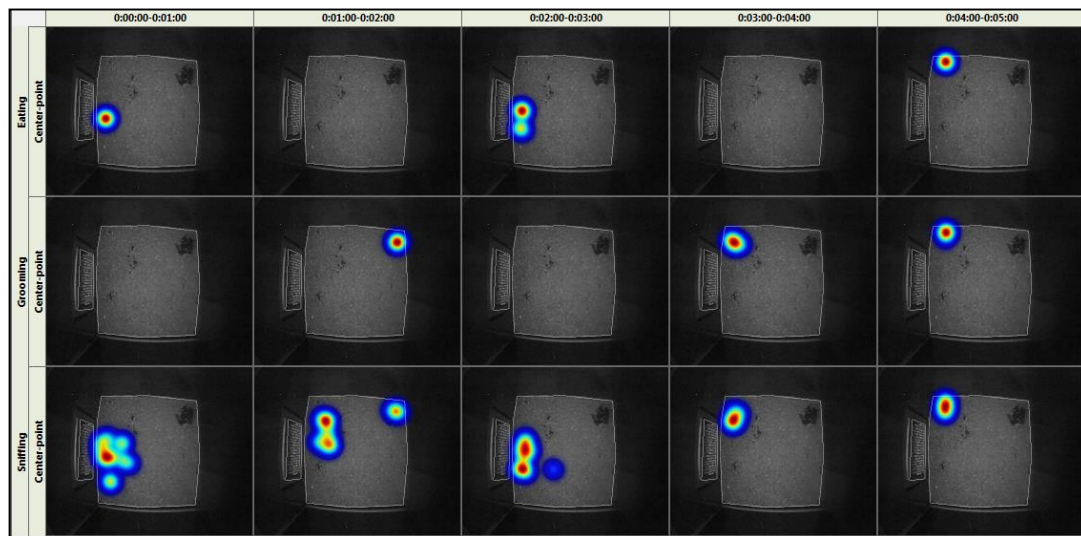
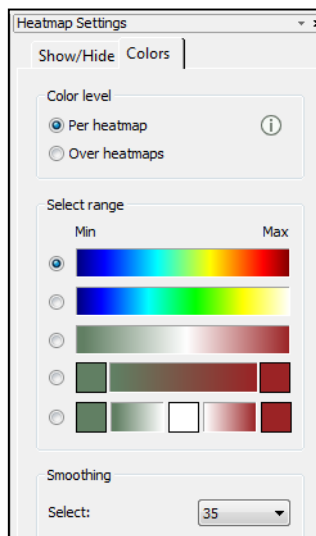
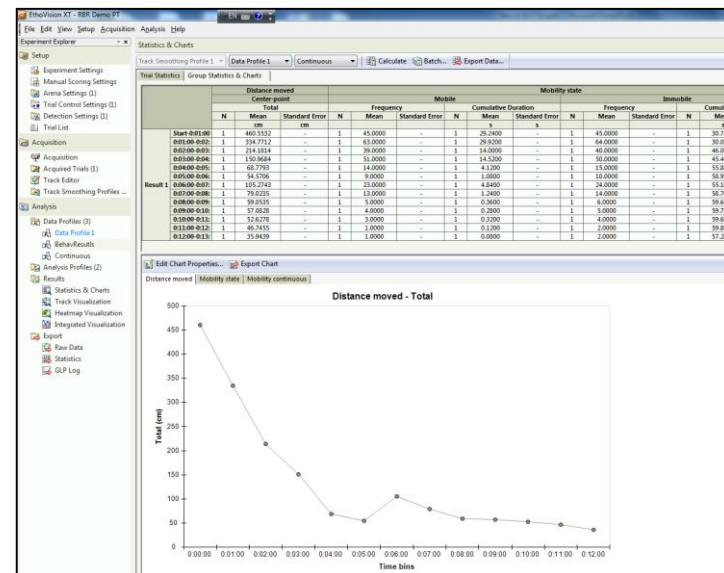
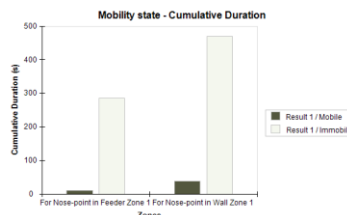
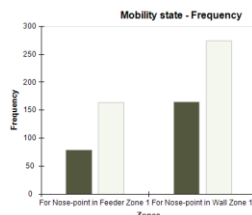
Interaction with devices

- Device events
- Device states

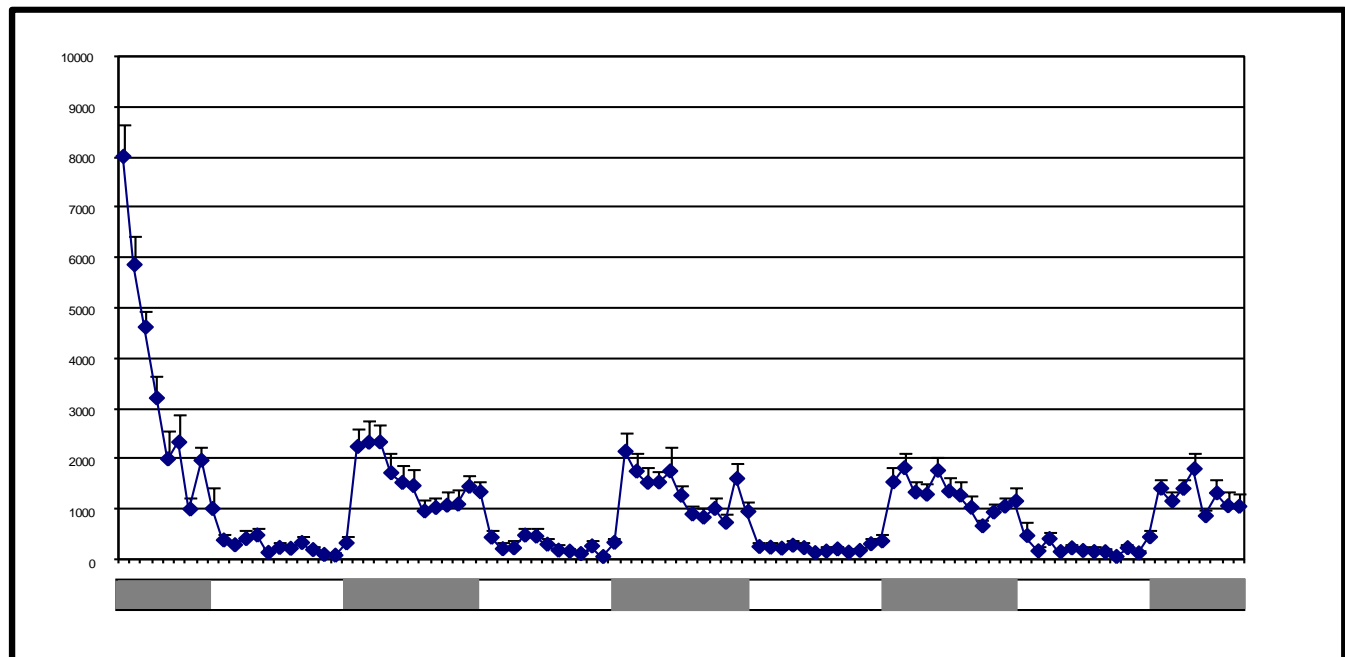
(*) Available for rat, under development for mouse

Graphs and charts

- Line charts
- Bar charts
- Heat maps
- Over time bins
- Over zones
- Over independent variables
- Publication-quality images

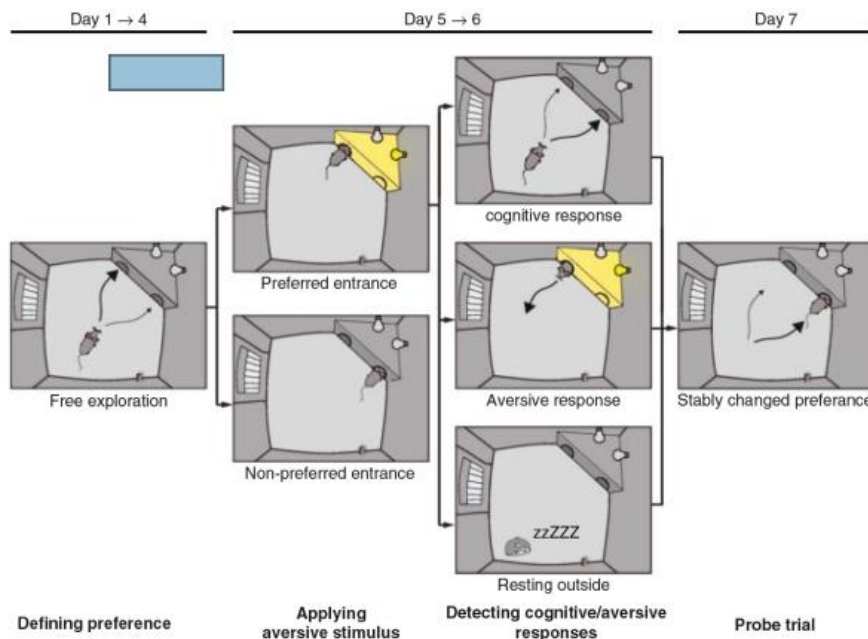


- Quantify biorhythm
- Detect (ab)normal sleep/wake cycle
- Schedule cognitive challenges during active phase



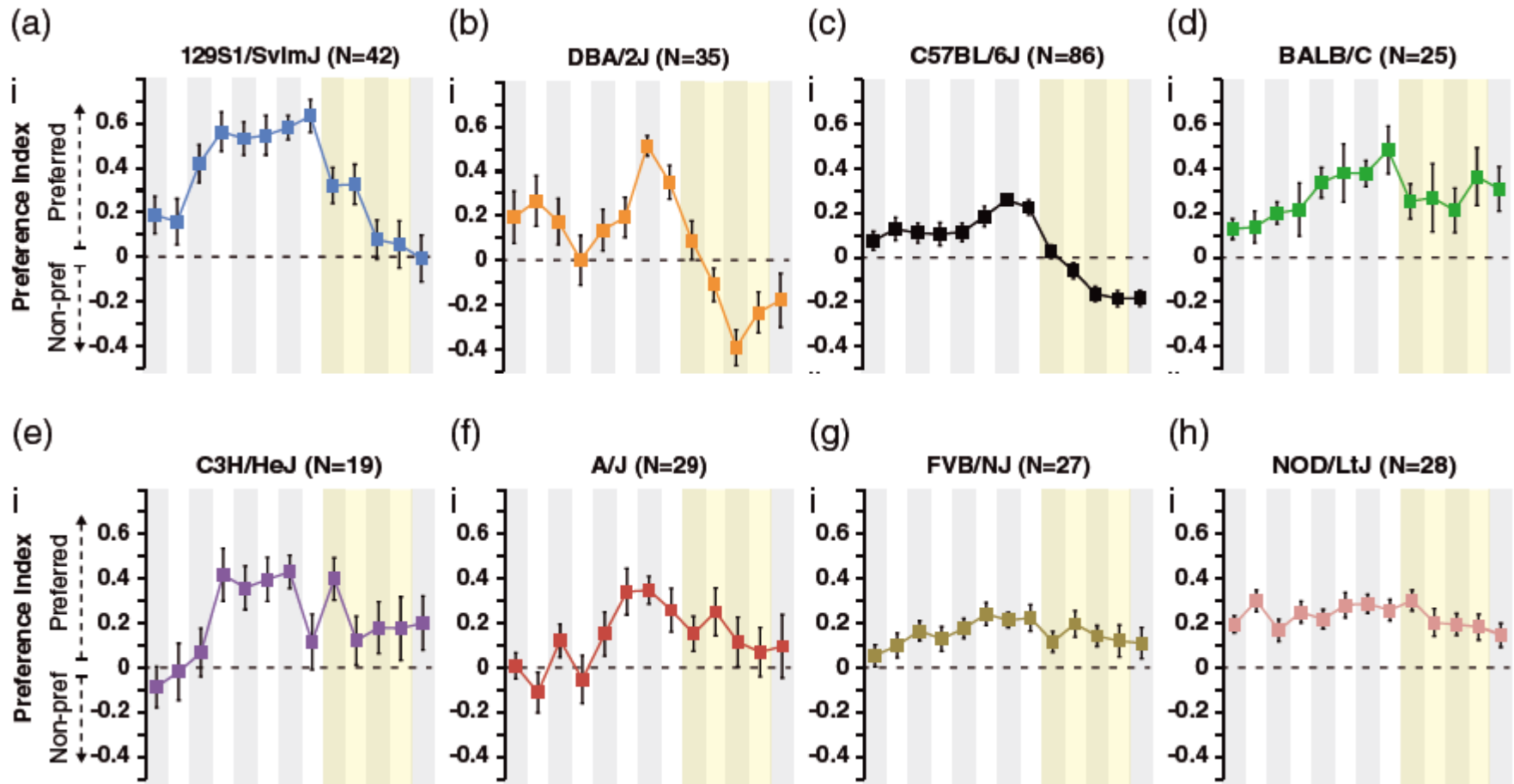
Locomotor activity (total distance moved) of male C57/BL6 mice

- Discriminative Avoidance Task in mice
 - Aversive stimulus: light shining into the shelter
 - Light switched on briefly or continuously depending on the presence of the mouse near or inside the shelter
 - Mice can learn to avoid the “preferred entrance” of the shelter
- Software control via EthoVision XT

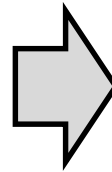
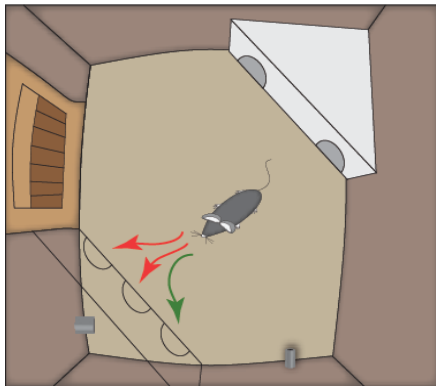


- De Heer & Spruijt (2008)
Proc. Measuring Behavior 2008.
- Maroteaux et al. (2012)
Genes, Brain and Behavior.

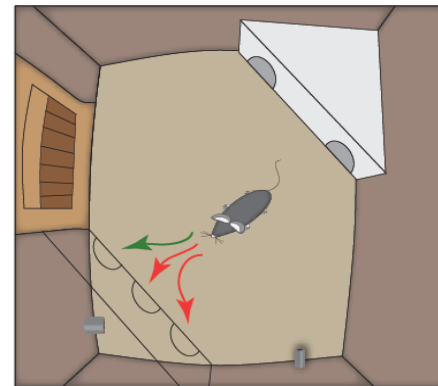
Avoidance learning: Results



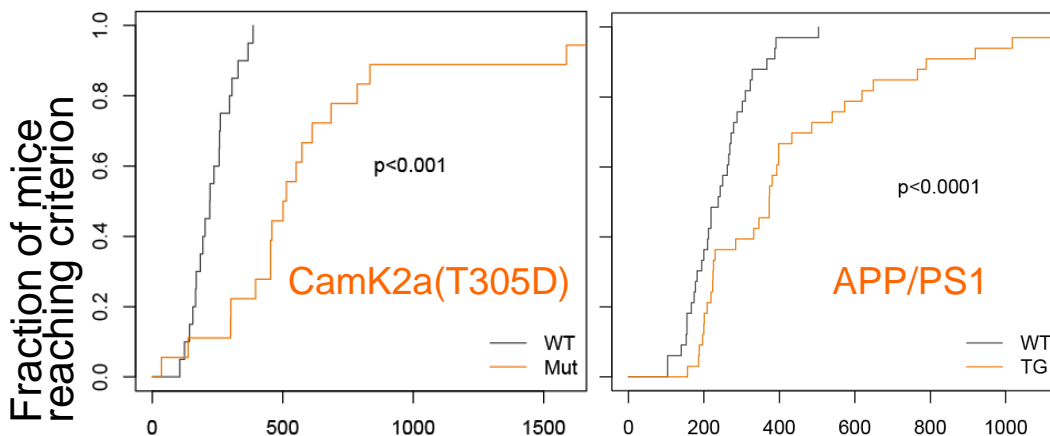
Discrimination learning (Left, 2 days)



Reversal learning (Right, 2 days)

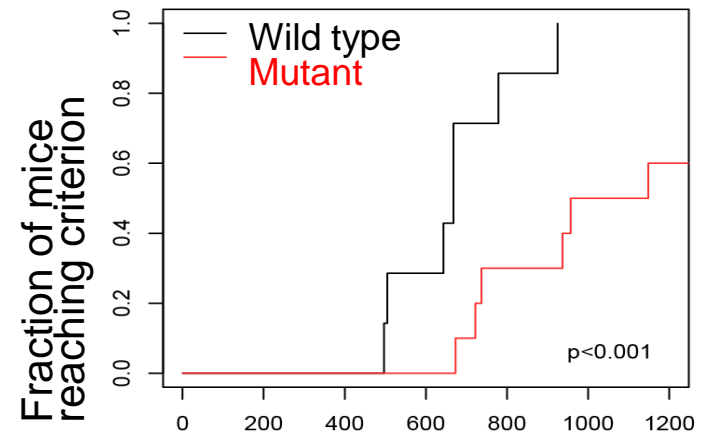


Example: **CamK2a(T305D)** and **APP/PS1** mutant mice require more entries to learn to discriminate

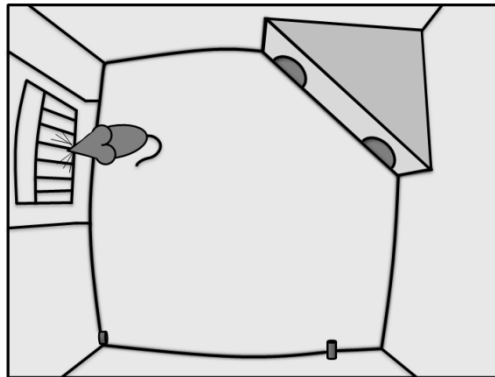


Entries required for **Discrimination learning**

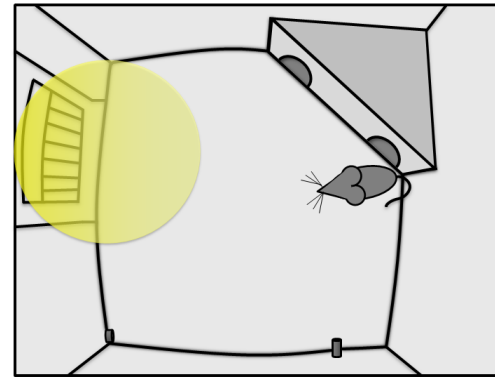
Example: A novel mutant mouse required more entries during reversal learning



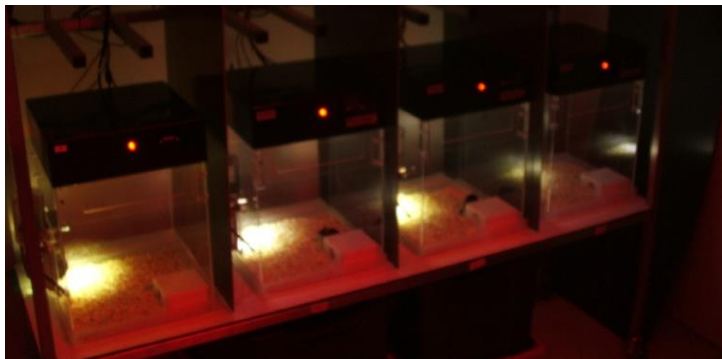
Entries required for **Reversal learning**



Normal behavior during the 1st hour of dark phase on a day preceding the anxiety test (**NoLightspot**)

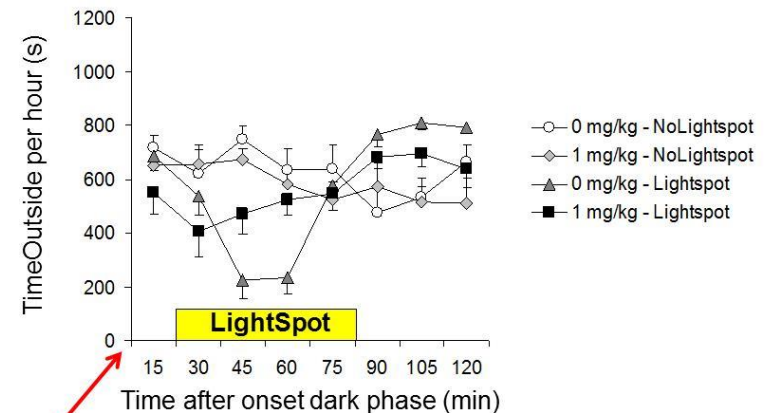


Avoidance behavior during the 1st hour of dark phase when the LightSpot is switched on (**Lightspot**)



Aarts *et al.*, in prep

Validation: Anxiolytic effect of diazepam



Oral administration @ 18:55

PhenoType cages, equipped with video camera, sensors and controllable stimuli



EthoVision

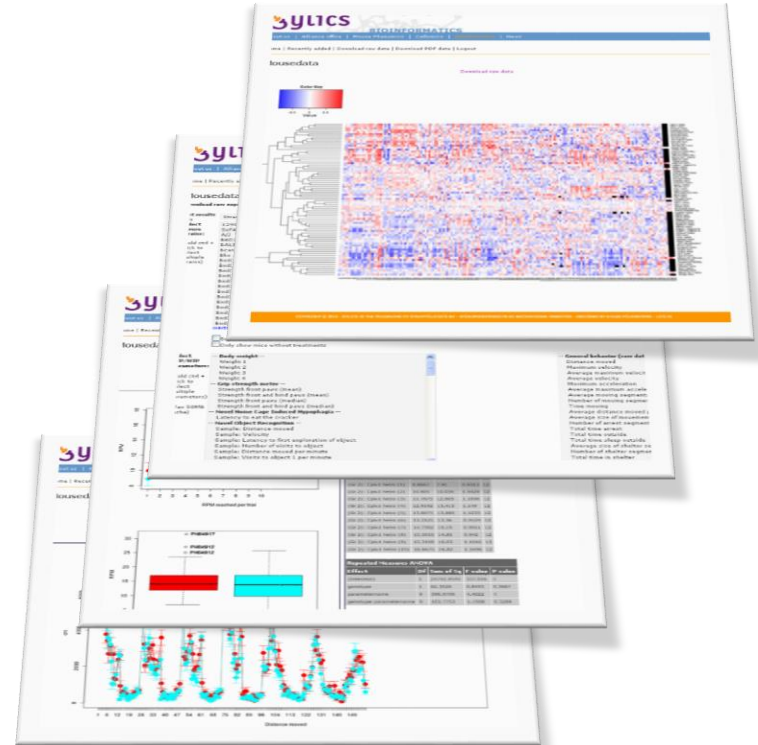


EthoVision software runs a **standardized testing protocol** by real-time video analysis and hardware control

AHCODA

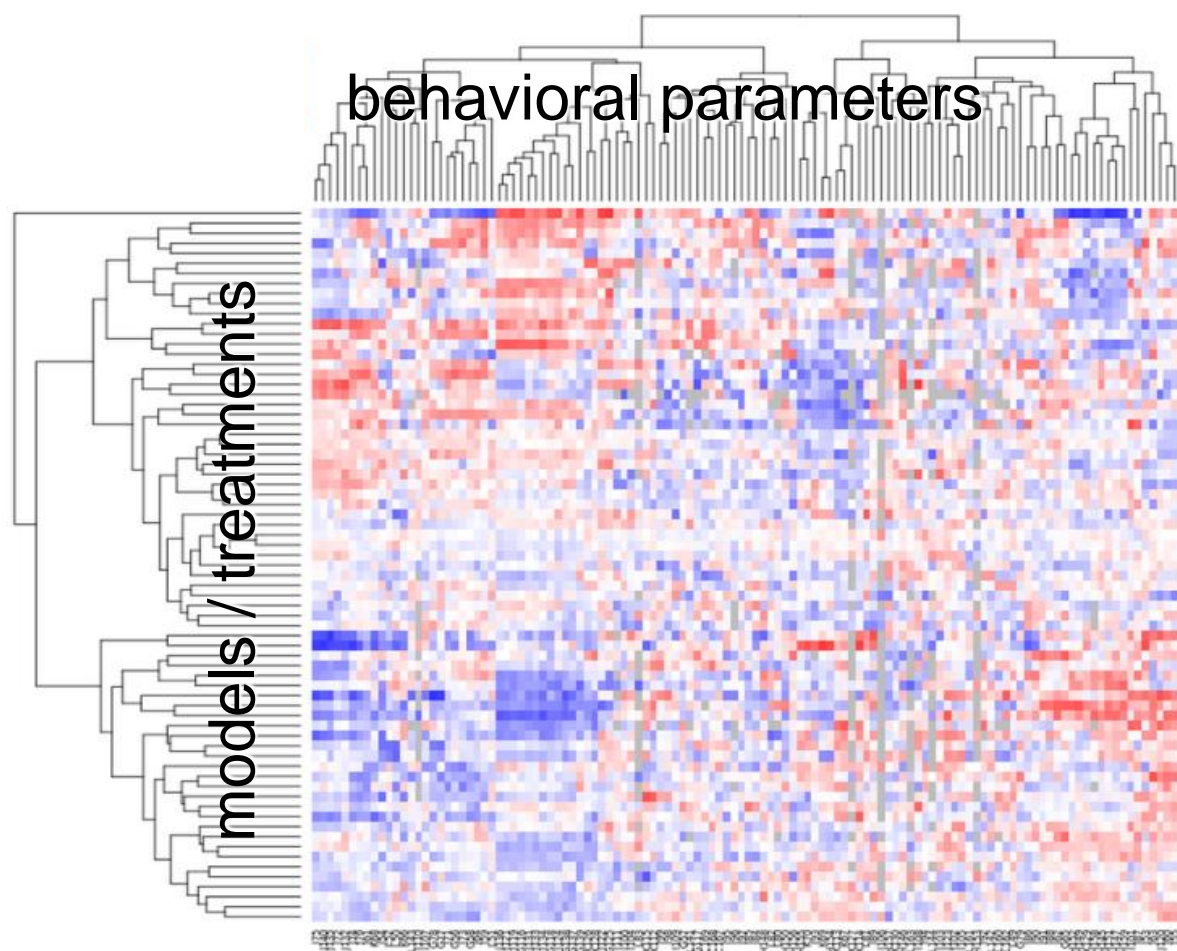
mousedata.sylics.com

Systematic integration, mining, and visualization of behavioral data in a web browser



AHCODA software processes **standardized testing protocol** data, performs QC, and adds the results to a cloud-based reference database within 24 h after behavioral testing

Mining for behavioral biomarkers and classification of models and treatments
Systematic comparison of novel models / compounds with existing profiles of models and compounds (>10,000 individual mice)



Popular disease models

AD: APP/PS1 and 5xFAD

PD: MTPT induced

Synaptopathies

ALS: SOD1 model

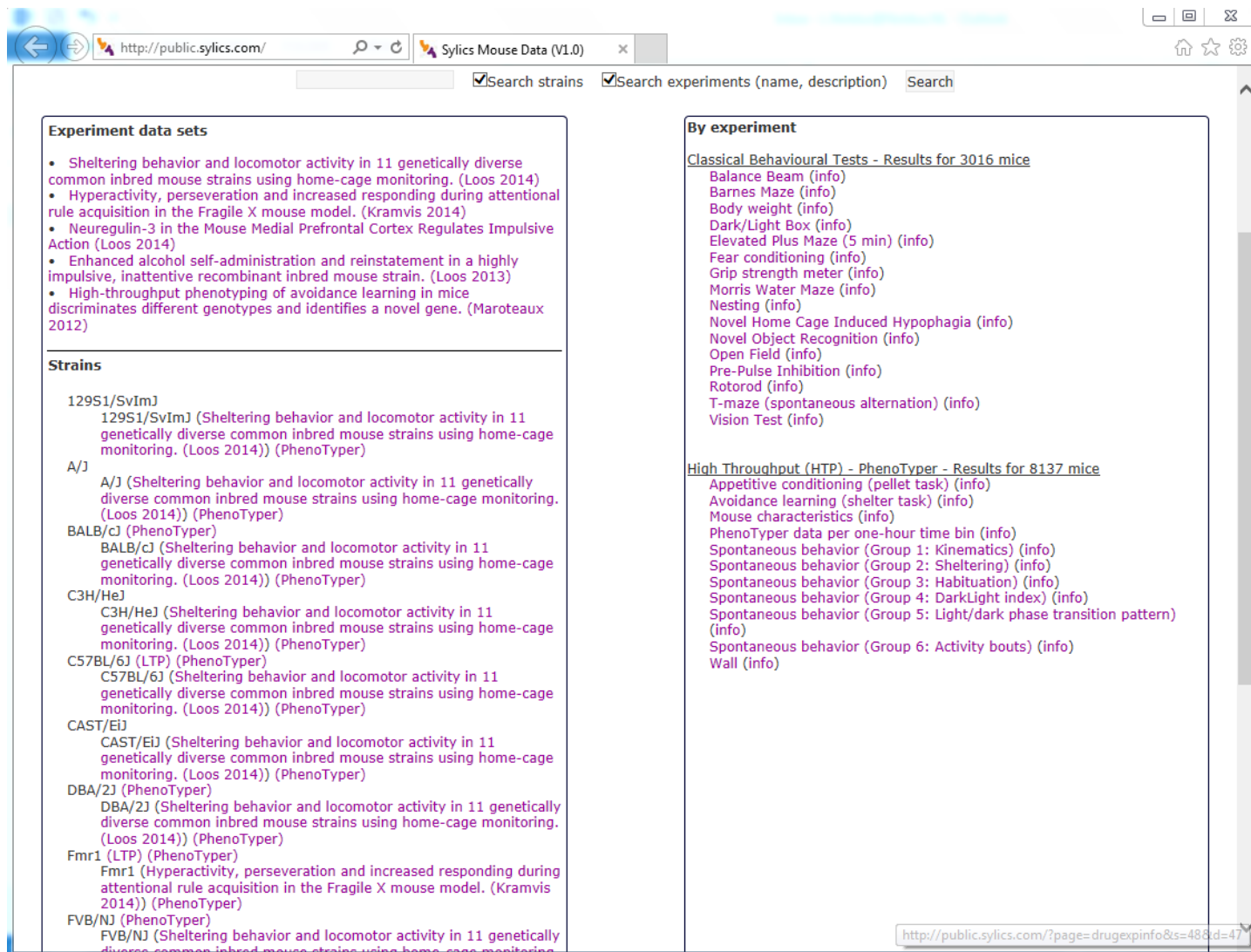
And many more...

Reference compounds

Diazepam

PCP

And many more...



The screenshot shows the Sylics Mouse Data (V1.0) website. The browser address bar displays <http://public.sylics.com/>. The page has a search bar with the following options: ☒ Search strains, ☒ Search experiments (name, description), and a Search button.

Experiment data sets

- Sheltering behavior and locomotor activity in 11 genetically diverse common inbred mouse strains using home-cage monitoring. (Loos 2014)
- Hyperactivity, perseveration and increased responding during attentional rule acquisition in the Fragile X mouse model. (Kramvis 2014)
- Neuregulin-3 in the Mouse Medial Prefrontal Cortex Regulates Impulsive Action (Loos 2014)
- Enhanced alcohol self-administration and reinstatement in a highly impulsive, inattentive recombinant inbred mouse strain. (Loos 2013)
- High-throughput phenotyping of avoidance learning in mice discriminates different genotypes and identifies a novel gene. (Maroteaux 2012)

Strains

129S1/SvImJ
129S1/SvImJ (Sheltering behavior and locomotor activity in 11 genetically diverse common inbred mouse strains using home-cage monitoring. (Loos 2014)) (PhenoTyper)

A/J
A/J (Sheltering behavior and locomotor activity in 11 genetically diverse common inbred mouse strains using home-cage monitoring. (Loos 2014)) (PhenoTyper)

BALB/cJ (PhenoTyper)
BALB/cJ (Sheltering behavior and locomotor activity in 11 genetically diverse common inbred mouse strains using home-cage monitoring. (Loos 2014)) (PhenoTyper)

C3H/HeJ
C3H/HeJ (Sheltering behavior and locomotor activity in 11 genetically diverse common inbred mouse strains using home-cage monitoring. (Loos 2014)) (PhenoTyper)

C57BL/6J (LTP) (PhenoTyper)
C57BL/6J (Sheltering behavior and locomotor activity in 11 genetically diverse common inbred mouse strains using home-cage monitoring. (Loos 2014)) (PhenoTyper)

CAST/EiJ
CAST/EiJ (Sheltering behavior and locomotor activity in 11 genetically diverse common inbred mouse strains using home-cage monitoring. (Loos 2014)) (PhenoTyper)

DBA/2J (PhenoTyper)
DBA/2J (Sheltering behavior and locomotor activity in 11 genetically diverse common inbred mouse strains using home-cage monitoring. (Loos 2014)) (PhenoTyper)

Fmr1 (LTP) (PhenoTyper)
Fmr1 (Hyperactivity, perseveration and increased responding during attentional rule acquisition in the Fragile X mouse model. (Kramvis 2014)) (PhenoTyper)

FVB/NJ (PhenoTyper)
FVB/NJ (Sheltering behavior and locomotor activity in 11 genetically diverse common inbred mouse strains using home-cage monitoring. (Loos 2014)) (PhenoTyper)

By experiment

Classical Behavioural Tests - Results for 2016 mice

- Balance Beam (info)
- Barnes Maze (info)
- Body weight (info)
- Dark/Light Box (info)
- Elevated Plus Maze (5 min) (info)
- Fear conditioning (info)
- Grip strength meter (info)
- Morris Water Maze (info)
- Nesting (info)
- Novel Home Cage Induced Hypophagia (info)
- Novel Object Recognition (info)
- Open Field (info)
- Pre-Pulse Inhibition (info)
- Rotorod (info)
- T-maze (spontaneous alternation) (info)
- Vision Test (info)

High Throughput (HTP) - PhenoTyper - Results for 8137 mice

- Appetitive conditioning (pellet task) (info)
- Avoidance learning (shelter task) (info)
- Mouse characteristics (info)
- PhenoTyper data per one-hour time bin (info)
- Spontaneous behavior (Group 1: Kinematics) (info)
- Spontaneous behavior (Group 2: Sheltering) (info)
- Spontaneous behavior (Group 3: Habituation) (info)
- Spontaneous behavior (Group 4: Dark/Light Index) (info)
- Spontaneous behavior (Group 5: Light/dark phase transition pattern) (info)
- Spontaneous behavior (Group 6: Activity bouts) (info)
- Wall (info)

The browser address bar at the bottom right shows the URL: <http://public.sylics.com/?page=drugexpinfo&s=48&d=47>

Spontaneous behaviors

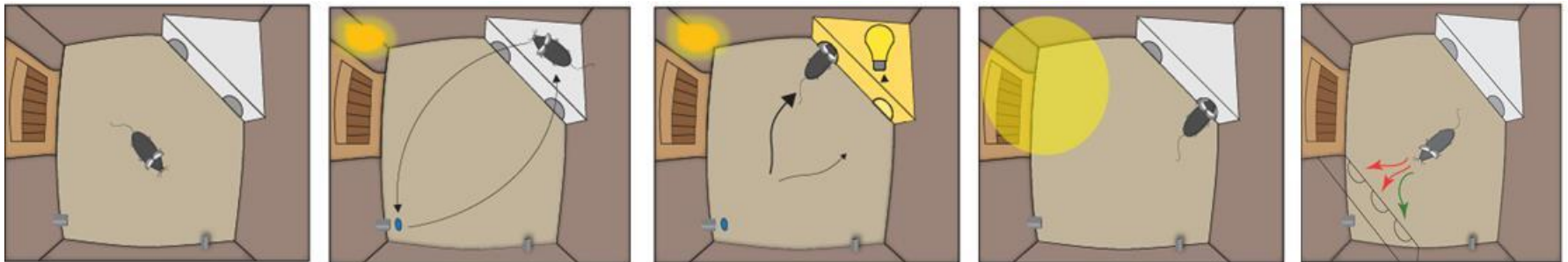
- Motor function
- Circadian rhythm
- Feeding pattern
- Novelty response
- Activity

Anxiety test

- Light avoidance

Cognitive tasks

- Avoidance learning
- Operant conditioning
- Reversal learning

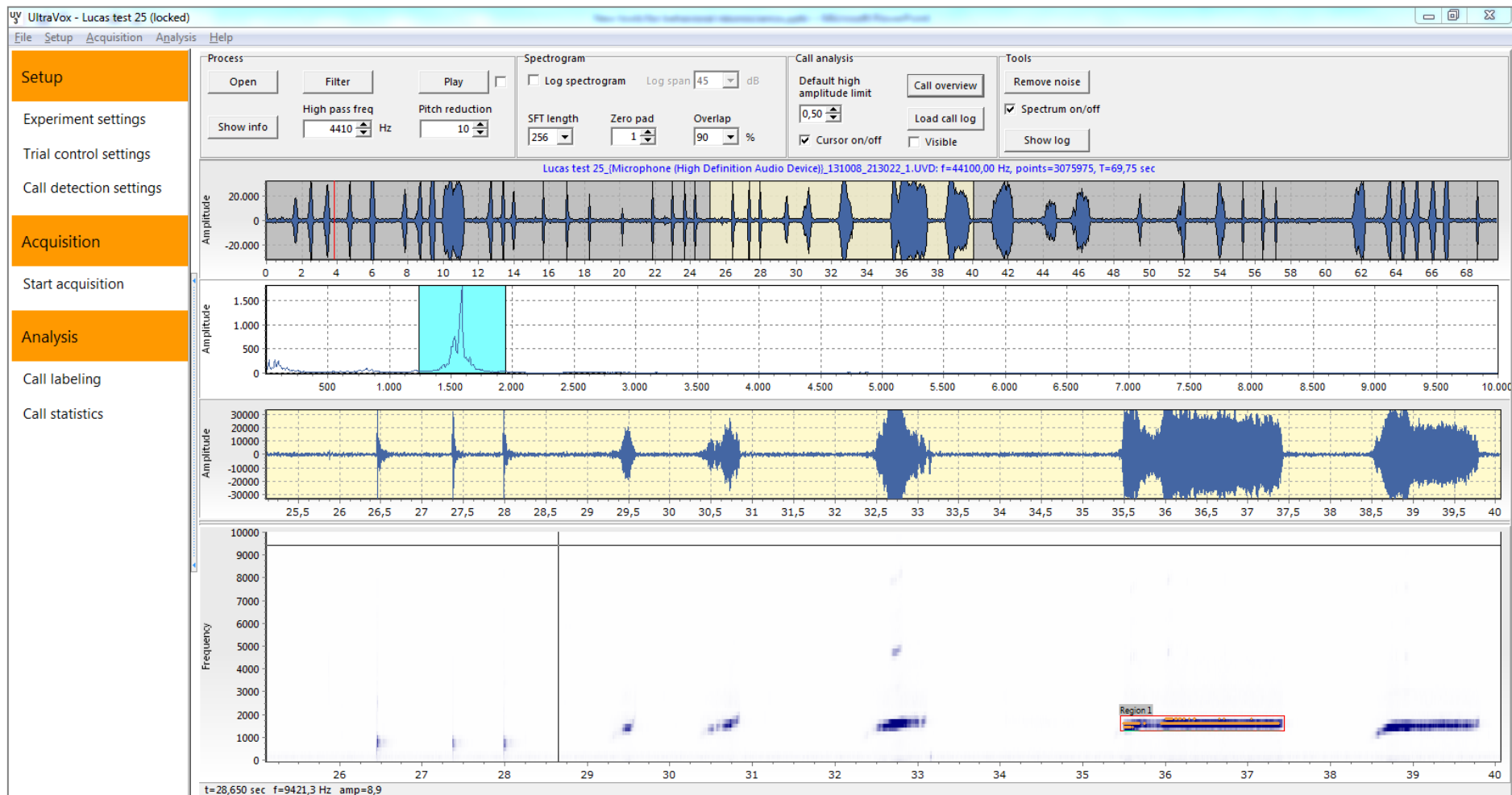


Under development:

- Detection of pain
- Working memory
- Impulsivity and attention
- Social interaction

- In use at ~125 laboratories
- NeuroBasic Mouse House (VU University Amsterdam):
more than 8,000 mice screened
- June 2014: **1,000th PhenoTyper** delivered to
The Jackson Laboratory





Fully automated system for screening motor performance and motor learning abilities of laboratory mice



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Any questions?