Novel Biomarkers, Animal Welfare and Data Reproducibility in the Home Cage

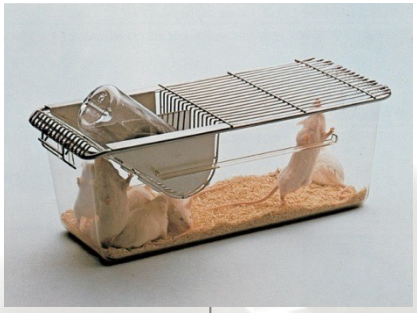
DIGILAB Marketing

TECNIPLAST

Tecniplast historical milestones in supporting science

1949 — Tecniplast foundation

1962 — The first moulded plastic cage



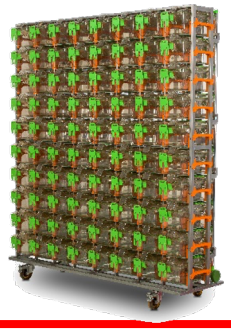
1992 — IWT a TECNIPLAST company

1994 — IVC first generation

1997 — Laminar Flow solutions

2005 — the Aquatic Division

TODAY — DVC® the Digital (R)Evolution



TECNIPLAST

Background motivations

(why a cage manufacturing company started this technological ambitious project)

The experimental use of animals is evolving

Mice spend **99%** of their time in the home cage

More active at **night**

Most of the experiments are performed during daytime

Enviromental factors (cage change, personell) are often underconsidered

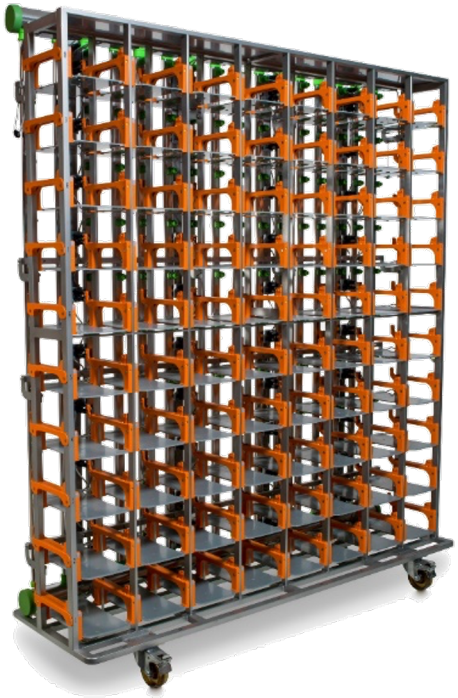
Cage Change effects, Weaning **effects longitudinal studies (genetics*age)**

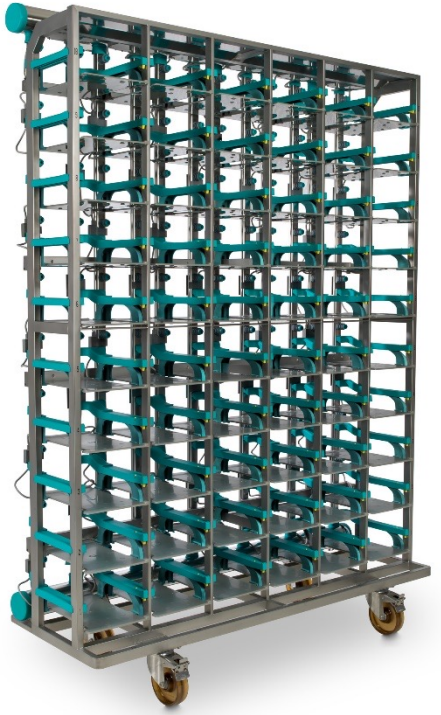
Locomotion/Activity behavioral test **are outside home cage**

Only Snapshot, What about Animal Welfare?

Our answer!

COLLECTING DIRECTLY INFO FROM THE TRUE HOME CAGE!!!





Tecniplast vision
Automated Home Cage Monitoring

Automated 24/7 data collection from the home cage provides several advantages:

- ✓ Keep animals in their home cage: **reduce animal handling**
- ✓ Allow **animals monitoring during periods** generally not observed (e.g. night time)
- ✓ **High throughput data**: several available IVC Cages running in parallel.
- ✓ Provide **standardized metrics**: just keep the animals in the home cage and automatically collect results (**reduced bias**).

DVC® VISION

Optimize and ease the facility management

Improve the quality of the reasearch

A recent feedback from the field:

*"Only by **combining animal welfare with scientific improvements** can we seriously address the **reproducibility** and **replicability** issues and the translational relevance of rodent phenotyping"*

Streamline Facility Management

- ***IMPROVED ANIMAL WELFARE*** – 24/7 monitor food and water availabilities, evaluate bedding condition and animal activity for a complete and **automated continuous animal welfare check**. Prevent any loss due to unexpected water floods.

- ***REAL TIME CAGE TRACKING SYSTEM*** – No more need to manually count your cages. DVC® features an **automated cage tracking system that in real time collects these data for you**: all the information you need for billing purposes are just one click away! Moreover, it provides clear reports regarding any cage history, it helps you to easily find cages and animals, as well as giving information regarding the real occupancy level of your animal rooms for a better logistic management.

- ***STREAMLINE YOUR JOB*** – The DVC® system **suggests when cage changes need to be performed**. This standardizes cage conditions and reduces animal stress, the number of cage changes as well as ergonomic issues related to repetitive actions, while cutting running costs and autoclave cycles. DVC® flexibility allows to customize the system accordingly to your needs and SOPs and **automatically daily balance your Vivarium workload to reach its maximum efficiency**.

Improve Research Quality

- ***OBTAIN NEW INSIGHTS*** – Thanks to automated 24/7 data collection, you will be able to capture **more information from subjects when they are most active** (e.g. in the night) without interfering with them.

- ***HIGHER AVAILABILITY OF DATA THROUGHOUT*** – Directly working at (DVC®) Rack Level **drastically improves number of available experimental samples** in terms of time (animals in their environment and no need to move into specific equipment for a limited time) and space (several available cage positions).

- ***EASE EXPERIMENTAL START-UP*** – No human intervention required to install experimental set up. **Simply house animals in the standard IVC cages in the DVC® rack**.

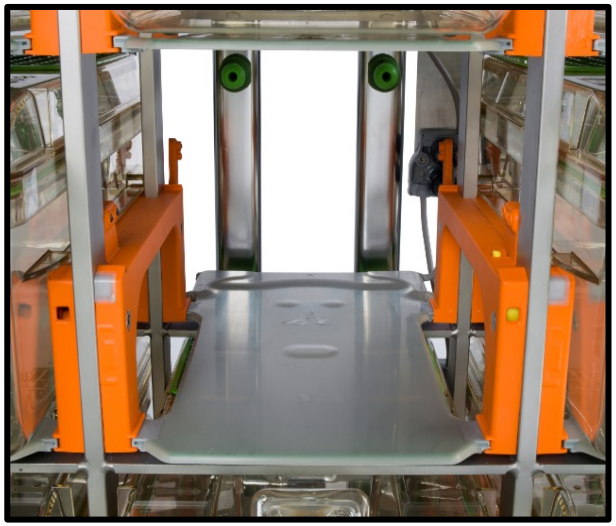
- ***INCREASE STUDY SENSITIVITY AND REPRODUCIBILITY*** – automatically collecting animal activity data, as well as related environmental conditions, **lead to deeper understanding of experimental outcomes and enhance study comparison**.

- ***MORE ROBUST DATA*** – automatically apply validated Tecniplast's metrics in parallel to all the experimental units without interfering with animals **leads to more robust and fully unbiased data**.

DVC® system

DVC
DIGITAL VENTILATED CAGE

- **Scalable Platform** (from 1 DVC® Rack to hundreds)
- **Elaborate real time** several sensors information about animal well-being, cage conditions and environmental factors

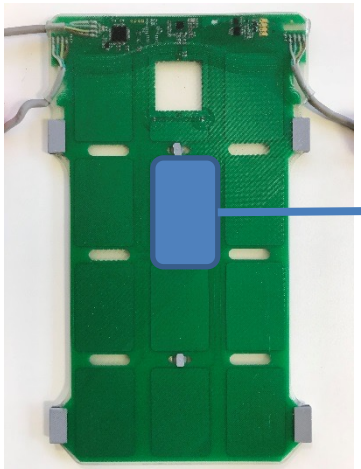


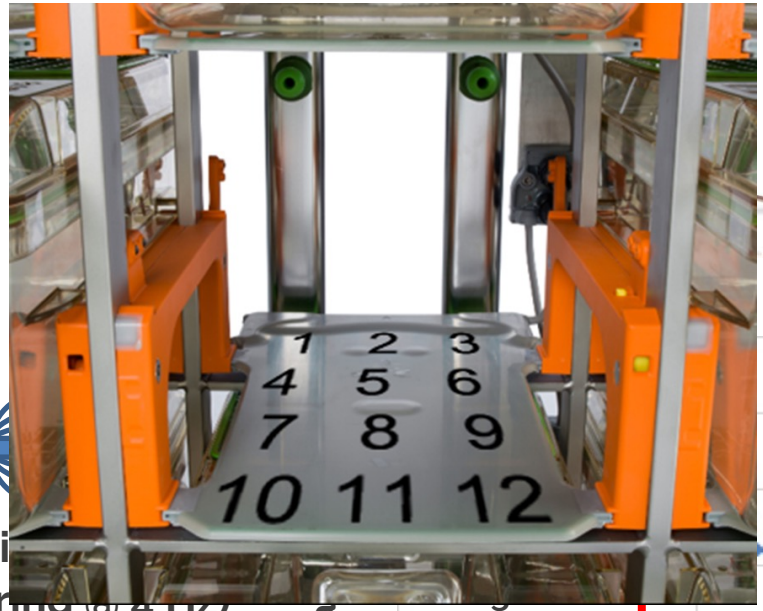
FULLY WASHABLE AND AUTOCLAVABLE (at 121°C / 250°F)

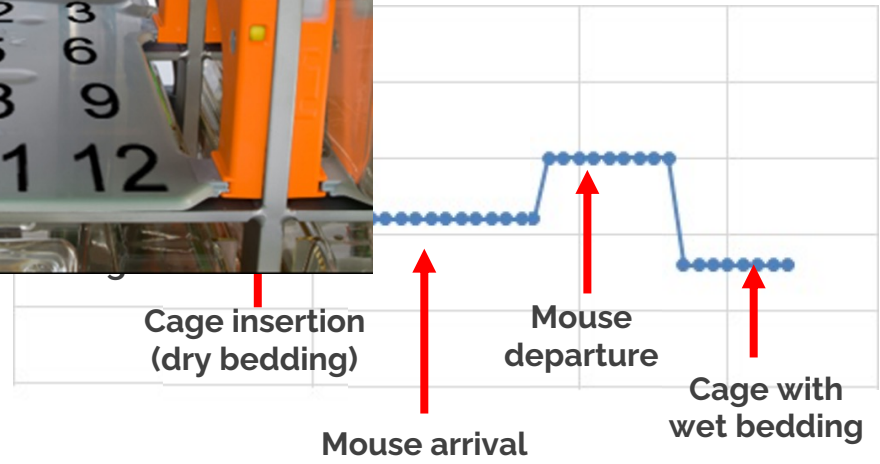
DVC® board

DVC® system working principle

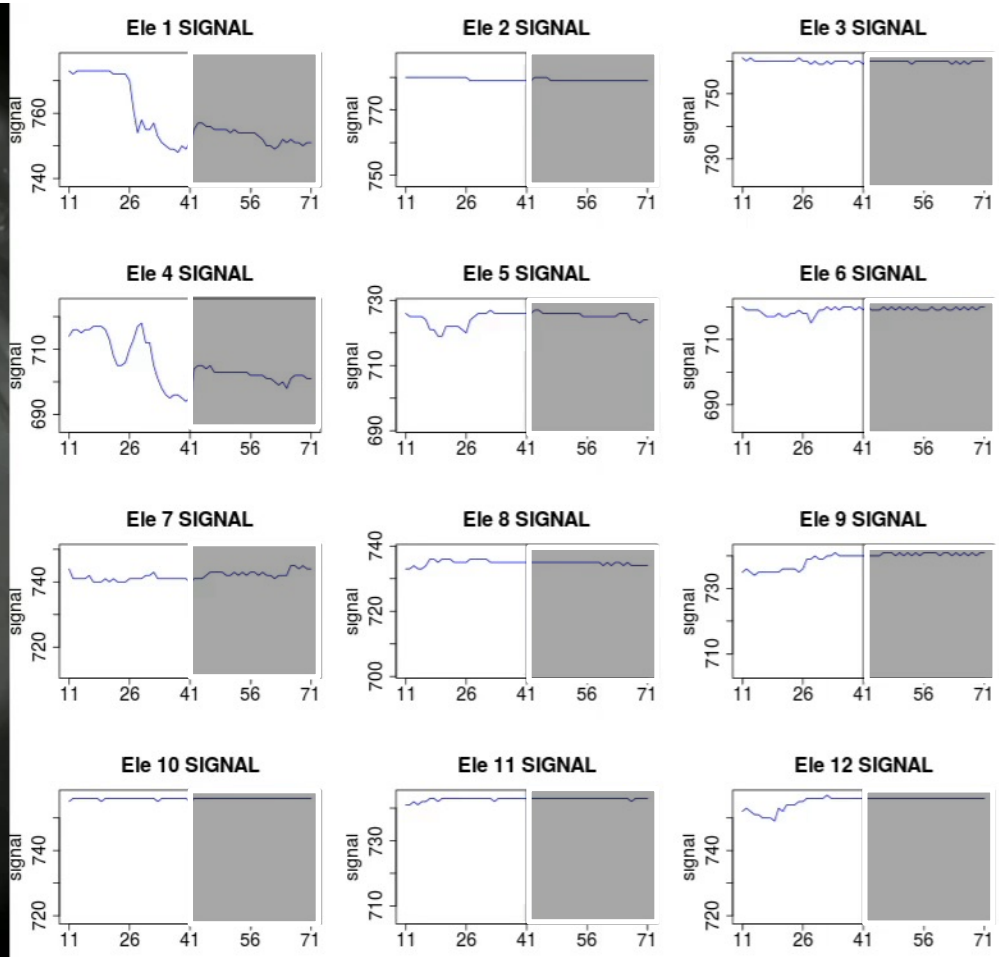
- The DVC® board is composed of **12 electrodes** a
- Each electrode acts as a proximity sensor (detects objects)





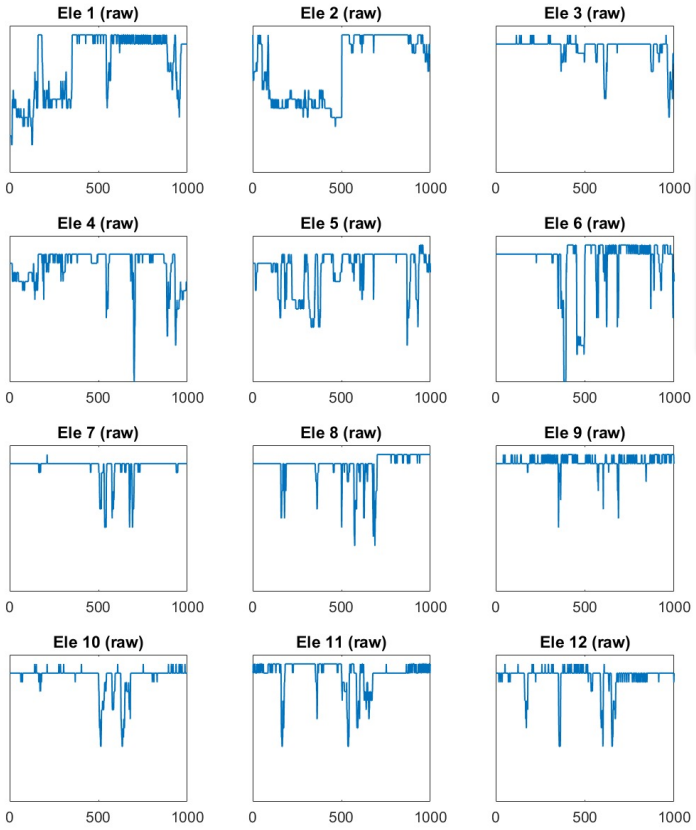


DVC® system working principle



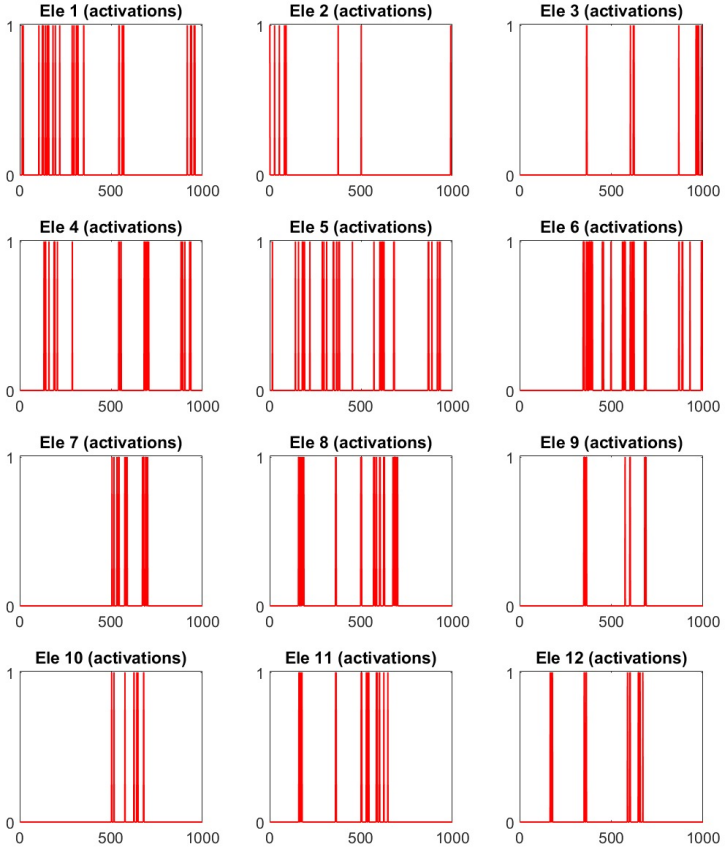
DVC® already validated metrics: *Animal Locomotion Index*

RAW DATA



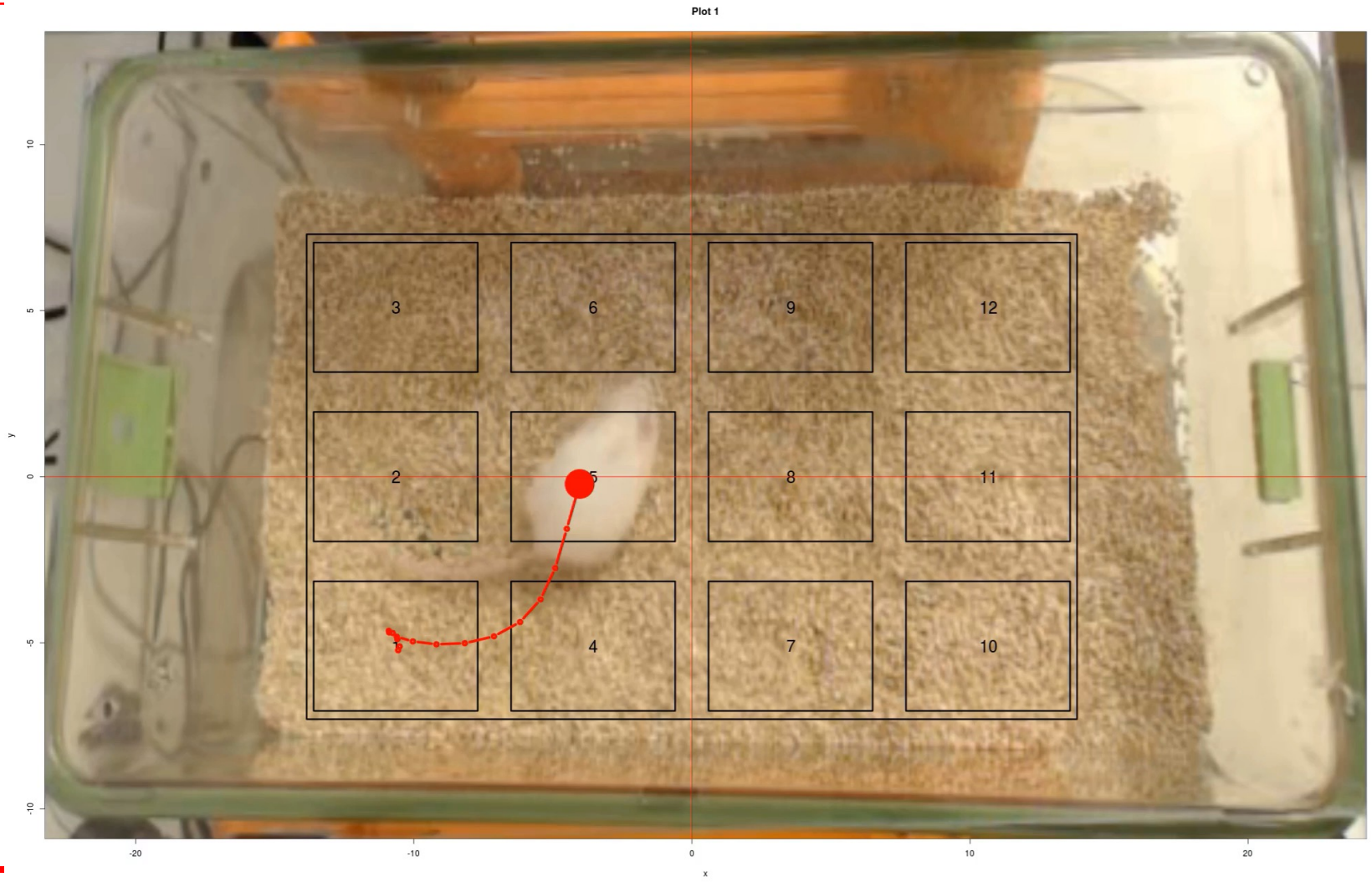
Electrode's capacity perturbations are translated into activations

METRIC



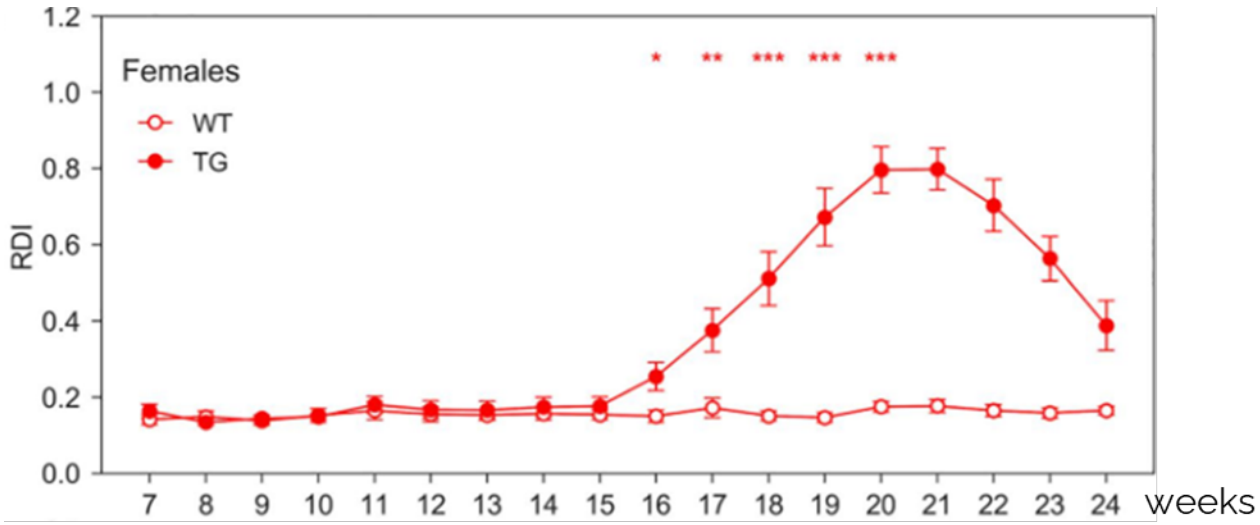
The corresponding data metric can be then grouped and displayed in different way to be analyzed

DVC® already validated metrics: *Individual mouse tracking*



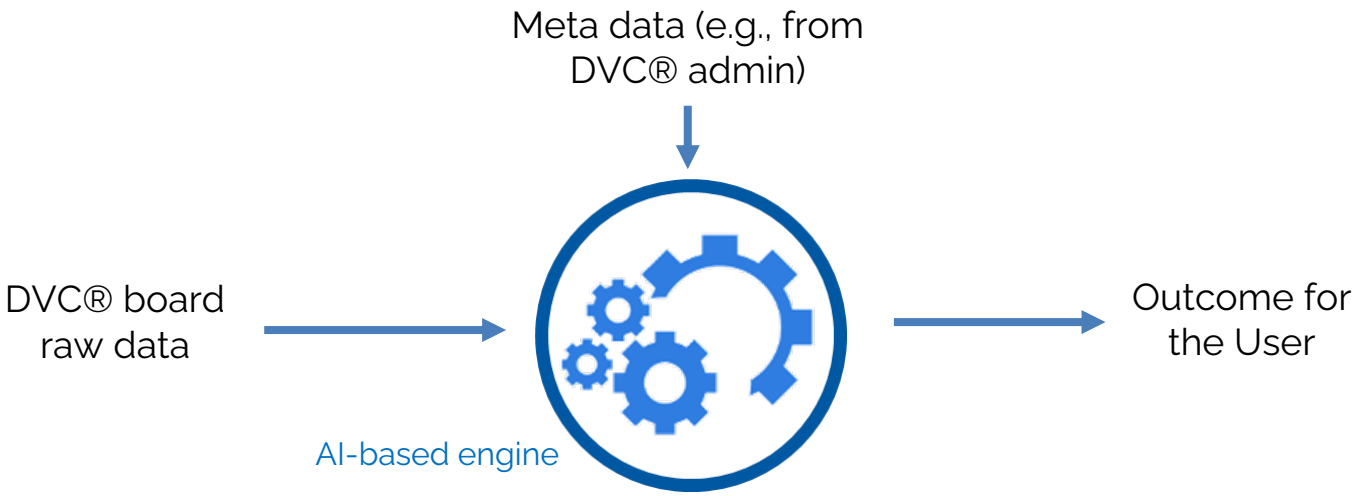
DVC® already validated metrics: *RDI*

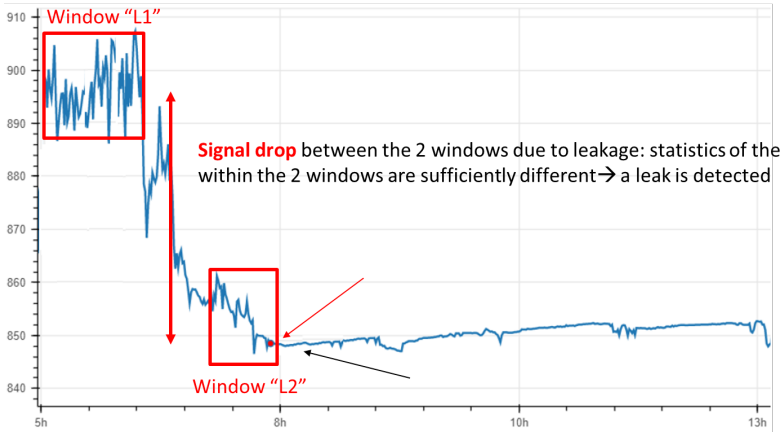
Regularity Disruption Index (RDI), which has been developed **to capture irregular animal activity patterns**.
To quantitatively capture these patterns, we designed RDI based on the **sample entropy** (Richman and Moorman, 2000) as the core metric.



RDI is a metric that measures irregularities of a time series (e.g., home cage activity), and it is not influenced by the absolute amount of the activity itself.

DVC® algorithms for the VIVARIUM



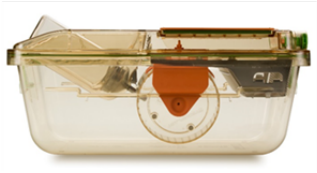


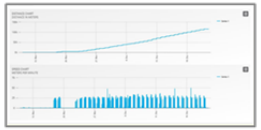
* LDS algorithm example

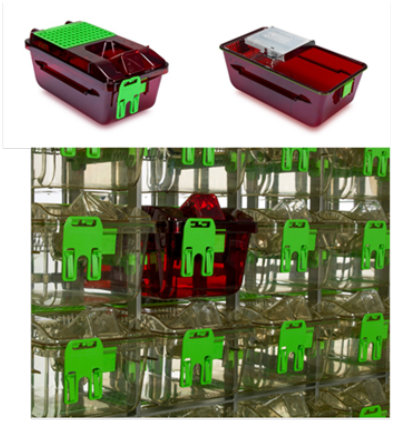
- Anomalous Animal Activity analysis
- Bedding Condition Analysis
- Food Availability
- Water Bottle Availability
- Water Flooding detection
- No activity from registered cages
- Activity from unregistered cages
- ...

Extra DVC® tools

DVC® Running Wheel to automatically detects rotations and provides reports







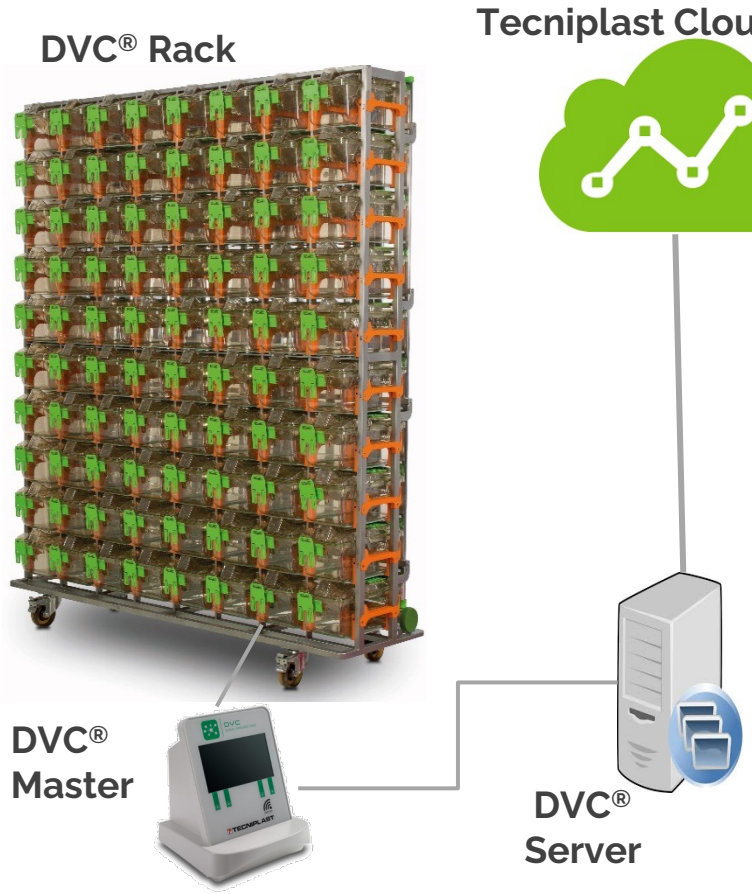
Leddy + Red/Black cage to change day-night cycle at cage level or simply standardize light conditions

DVC® Analytics for the RESEARCHERS

DVC® Analytics is the scientific **cloud-based** portal designed to provide full and easy access to all DVC® data and validated metrics

DVC™ ANALYTICS
SCIENTIFIC PLATFORM

DVC® Analytics for the RESEARCHERS



DVC® Analytics collects information from:

- **The DVC® Master**, containing animal behavioral indicators (i.e., **metrics)** calculated based on the raw data.
- **The DVC® server**, containing **metadata** information such as "cage id", "research protocol", "owner" and all the "cage events" (e.g., bedding change, treatments, etc.).

BENEFIT: All information are merged together, aggregated per "cage id" and can be displayed by cage id, cage group or can be compared by cages or groups!

1 DVC80 Rack generates around 10GB/month

DVC® Analytics for the RESEARCHERS

DVC® provides new opportunities

Automated 24/7 data collection from the home cage provides several advantages:

- ✓ Keep animals in their home cage: **reduce animal handling.**
- ✓ Allow **animals monitoring during periods** generally not observed (e.g. nighttime)
- ✓ **High throughput data**: all experimental cages running in parallel at the same time.
- ✓ Provide **standardized metrics**: just keep the animals in the home cage and automatically collect results (**reduced bias**).
- ✓ **Easy set up**: simply place animals in their home cages

DVC®: the tool to detect novel insights

Events affecting animal welfare

Laboratory animals live in a controlled environment which has the final goal of avoiding (reducing?) possible adversative events causing loss of animals, welfare issues or more general unusual behaviors that lead to wrong scientific interpretations and outcomes.

What is the real effect of a water flooding?

A very common issue occurring in any Animal Facility around the world is usually the water flooding due to leakages of bottles or AWS valves.

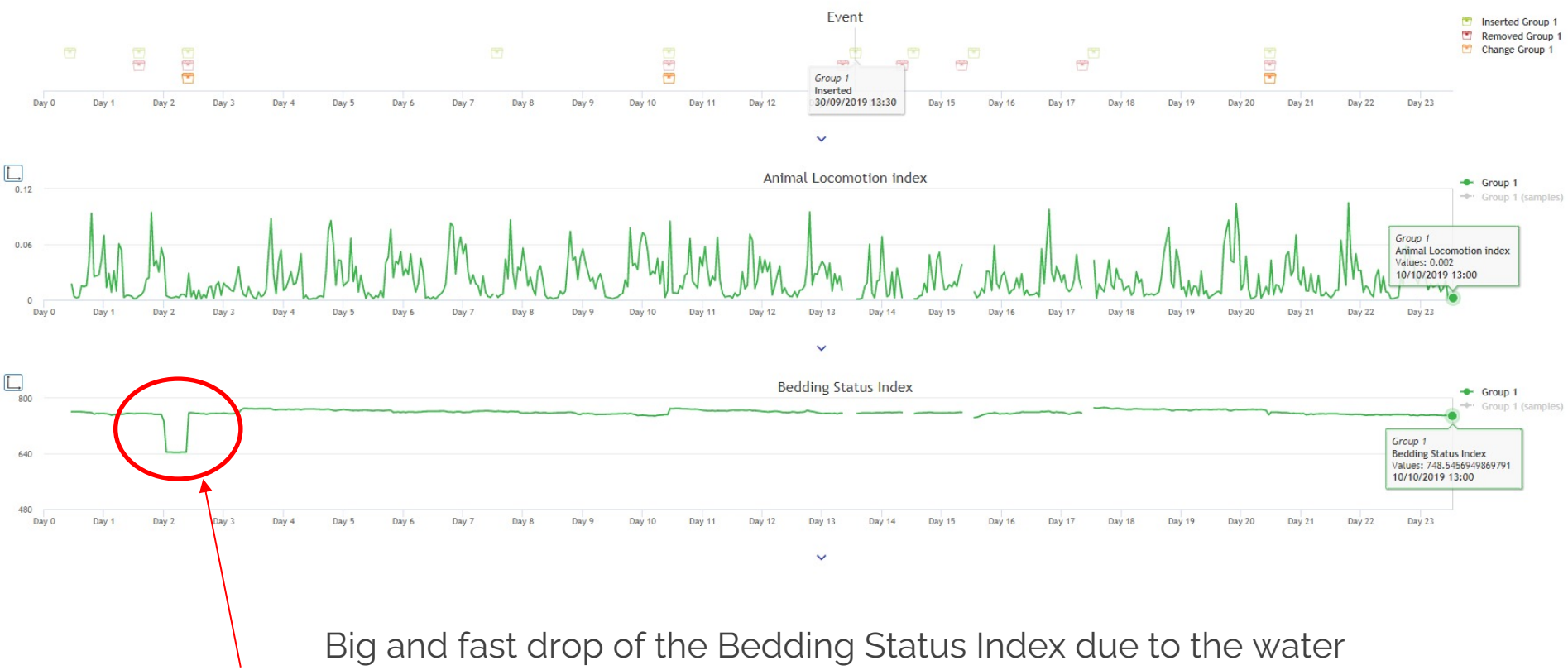
Everyone would like to avoid this scenario because can be fatal for animals (especially when AWS fails). But have you ever considered which are the side effects and how long they last ?

Water bottle flooding effect

In the next example, we reported a cage with **3 mice (C57BL/6J)** which had a **water bottle flooding** occurring in the middle of the night (probably due to animal drinking).

- 1st Line graph shows the global animal activity in the cage along a period of 23 days (from 17th Sept to 10th October 2019)
- 2nd Line graph shows the status of the bedding in terms of moisture in the same period.

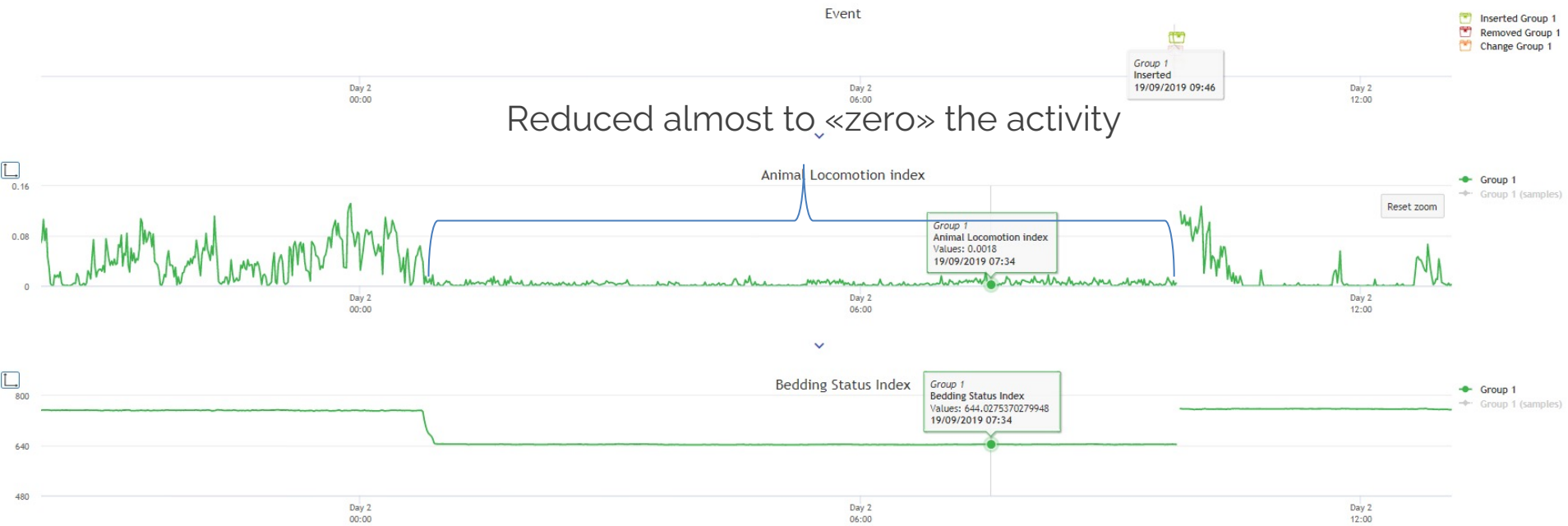
Water bottle flooding effect



Big and fast drop of the Bedding Status Index due to the water flooding (17° Sept at 01:32am)

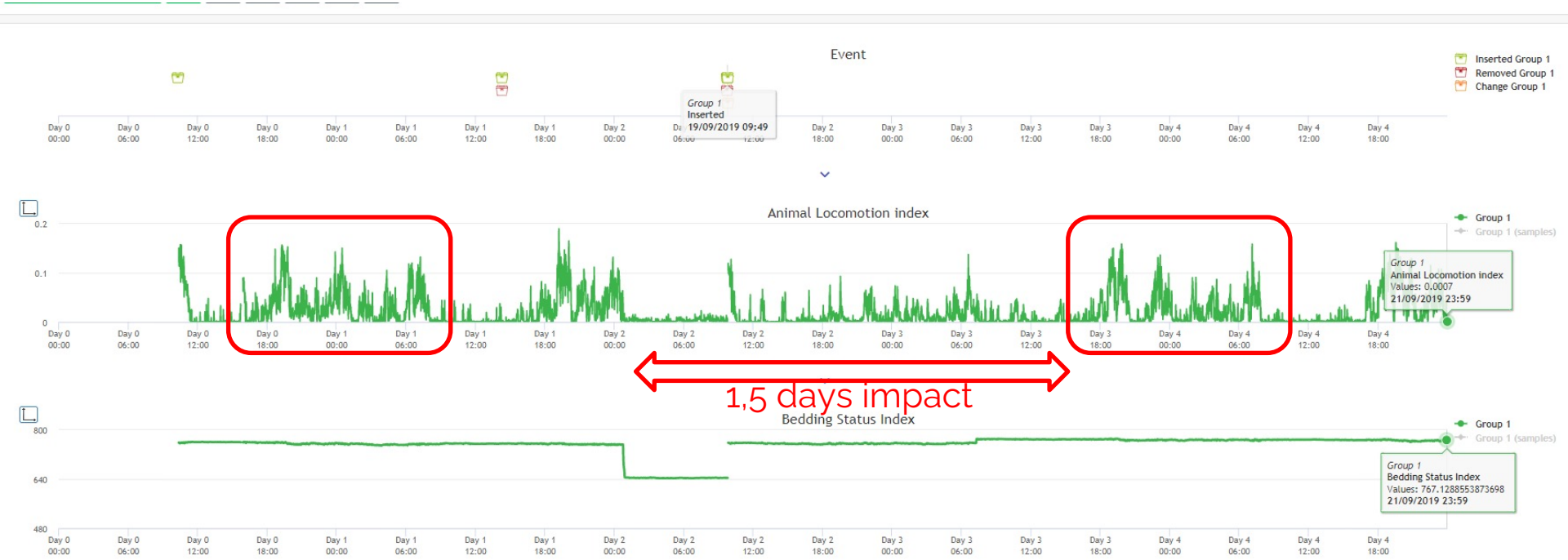
Water bottle flooding effect

Zooming the period and using a more detailed minute aggregation visualization in the DVC® Analytics we can see that, immediately after the water bottle flodding occured, the animals dramatically reduced their spontaneous locomotion around the cage until the cage itself was «rescued» by operators in the morning (changed)



Water bottle flooding effect

Now, looking at a wider temporal period including 2-3 days **after** the water flooding, it is extremely evident how much time the animals needed to really fully recover. The entire day (light) and night after the event, although now the cage was already back to «standard» conditions, shows that in reality the animals were still «under shock/stress» because the circadian rythm not yet fully recovered (in comparison to days before the event).



Improve the acclimation period knowledge

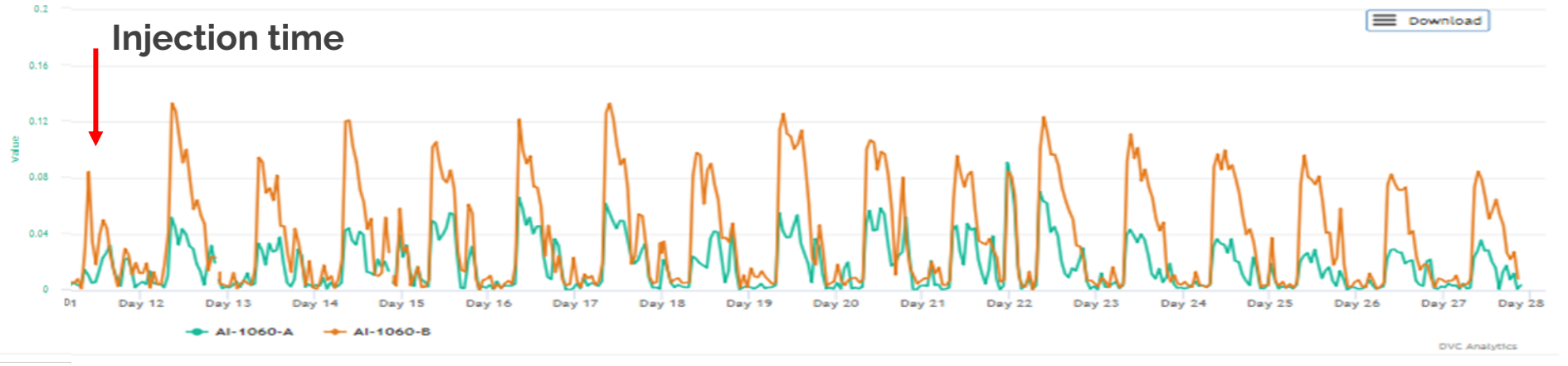
A researcher ordered some animals (**same strain, sex and age**) from a breeder which were shipped in 2 different moments. This fact led to create the first group of cages as control cages and the second ones as experimental group in 2 different moment of the day.

The design of the experiment required **10 days of acclimation** in the novel cages and then, only experimental animals have been injected with a specific compound able to generate hyperactivity in the experimental animals (control animals injected with saline solution).

The researcher was intrigued by the DVC® system because it is possible to detect animal activities especially during dark periods.

Improve the acclimation period knowledge

Thanks to the DVC® Analytics it is possible to group different cages and perform visual comparisons between groups to immediately analyse the results



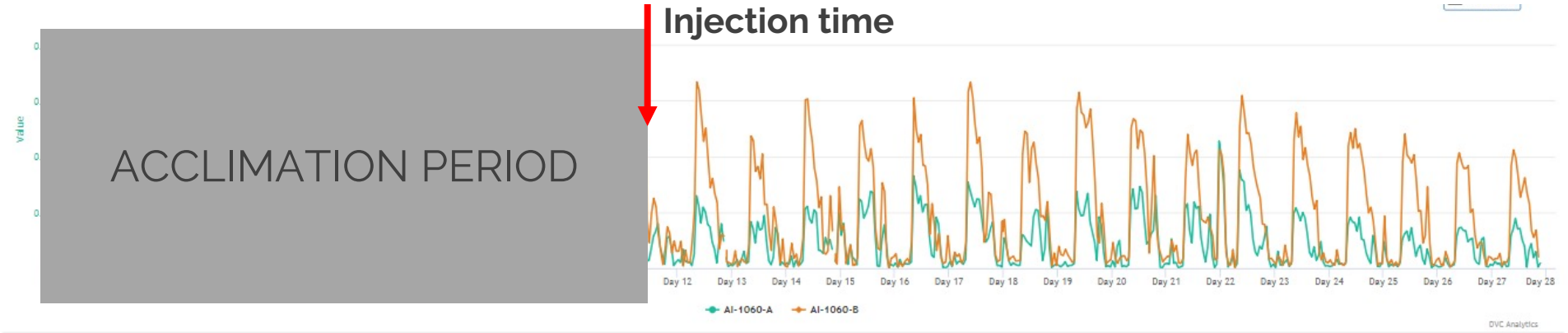
Researcher was incredibly happy to «see» the effect of the compound especially during night (not so evident during light phase). Thanks to the DVC® it was possible to easily validate the hypotesis…

BUT WHAT ABOUT THE ACCLIMATION PERIOD ???

Improve the acclimation period knowledge

All the cages were inserted into the DVC® system at day -10, So also for the acclimation period…

ACCLIMATION PERIOD



The 2 groups were already «different» at time of arrival!!!

Study example # 1: Towards Automated Home Cage Monitoring

RESEARCH ARTICLE

Towards large scale automated cage monitoring – Diurnal rhythm and impact of interventions on in-cage activity of C57BL/6J mice recorded 24/7 with a non-disrupting capacitive-based technique

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Study example # 1: Towards Automated Home Cage Monitoring

- **Multicentric study** carried out at:
  - Karolinksa Institute (Sweden)
  - Jackson Laboratories (USA)
  - CNR (Italy)

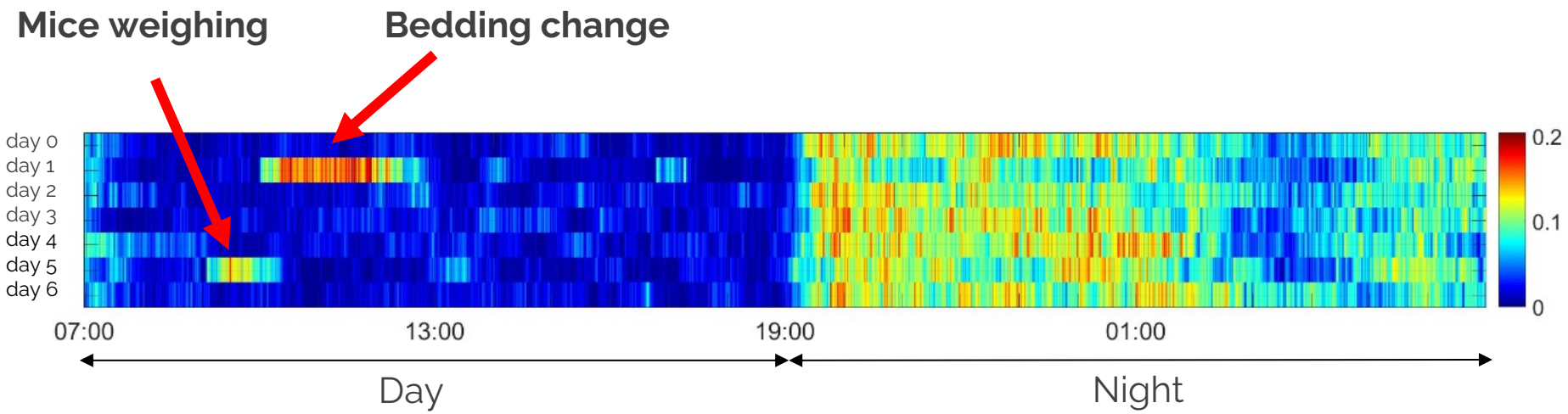
- **Experimental settings:**
  - Each site 5 cages
  - 5x ♀ per cage (Karolinska also ♂)
  - Once a week cage change

Hypotheses:

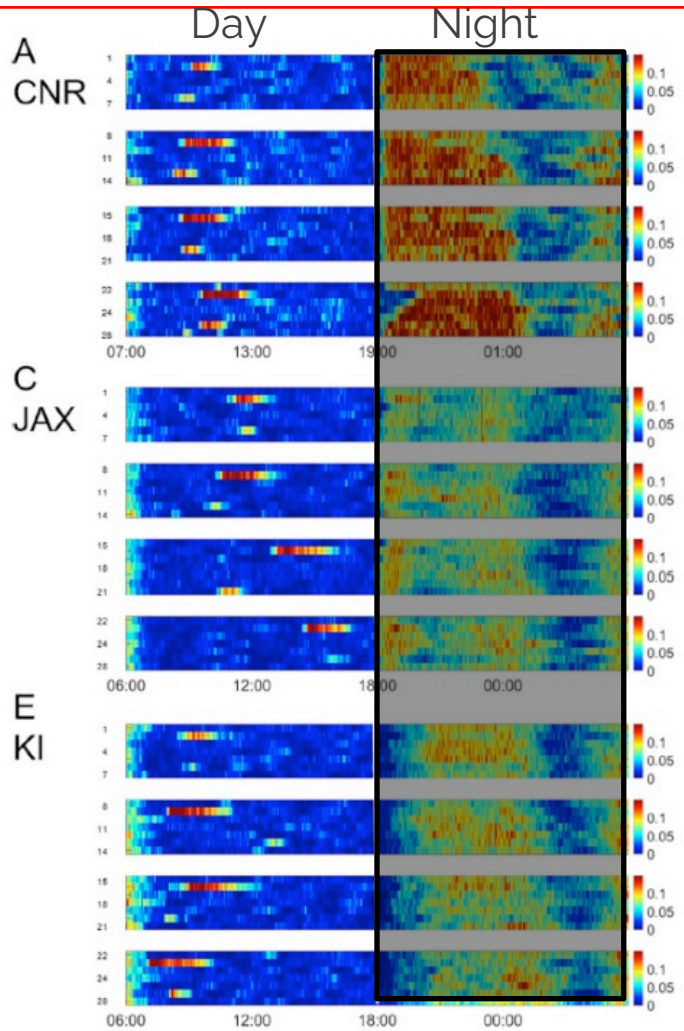
- Assess DVC® capabilities to monitor 24/7 home-cage animal activity behaviours
- Explore, investigate and quantify activity patterns (expected and unexpected)

Study example # 1: Towards Automated Home Cage Monitoring

- **DVC® metrics allow to identify and quantify**, e.g.:
  - effects of procedures such as:
    - bedding changes
    - Weighing
    - Lights ON/OFF
  - Circadian rhythm
  - Floor areas activity preference

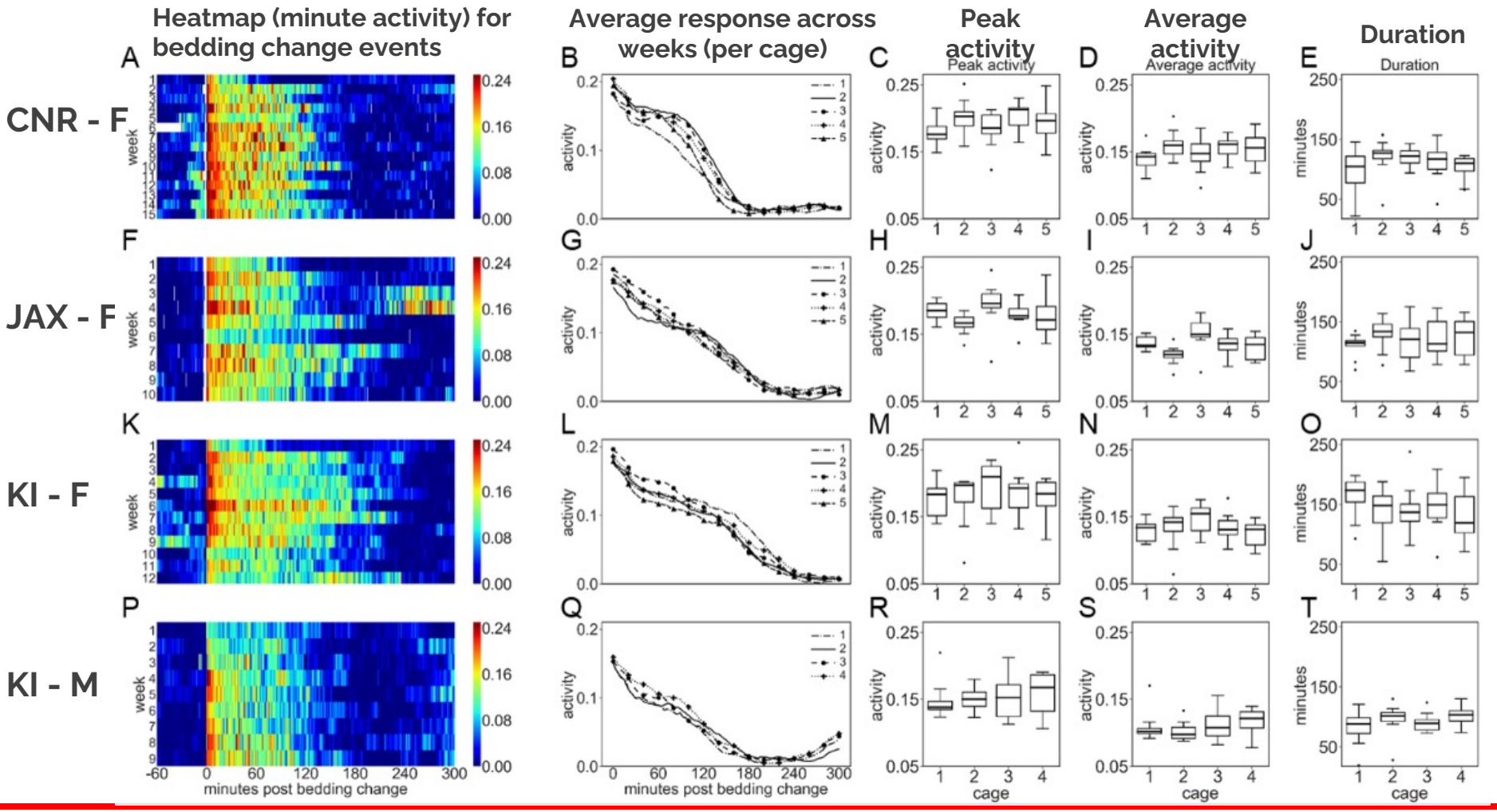


Study example # 1:
Towards Automated Home Cage Monitoring



- Clear diurnal difference in activity
- Difference at night time among sites (enviromental factors)

Study example # 1:
Towards Automated Home Cage Monitoring



Study example # 1: Towards Automated Home Cage Monitoring

Study conclusions:

- Clear difference among sites despite same strain/breeder
- Cage change stressor could unveil responses in lame animals➔ emotional response, arousal facilitate differences to be identified.

Study example # 2: ALS progression in transgenic SOD mice

Goal of the experiment:

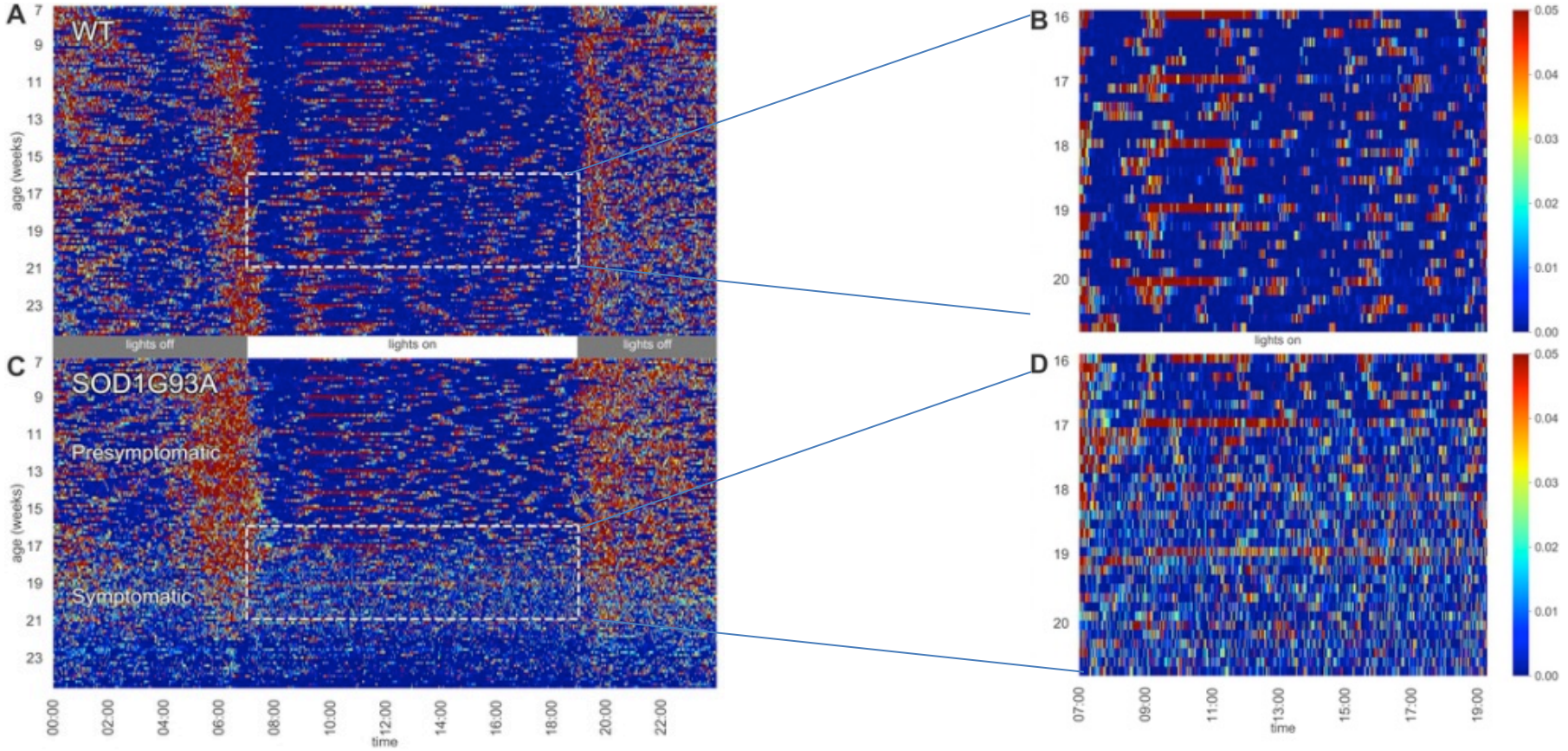
- **Assess** DVC® capabilities to detect **ALS progression over time** (ALS-related locomotion impairments symptoms in SOD mice appear at ~16 weeks of age) vs. standard behavioral test (e.g., grid test)

- **Experimental settings:**
  - 10 cages WT and KO male, 16 cages WT and KO female
  - 2x mouse/cage
  - > 4 months mice observation via DVC®
  - 1x week cage change

  - Behavioral Test Grip Test, Grid Test➔ Sign of muscular Atrophy

Study example # 2:
ALS progression in transgenic SOD mice

Qualitative assessment of activity: Heatmap



Study example # 2:
ALS progression in transgenic SOD mice

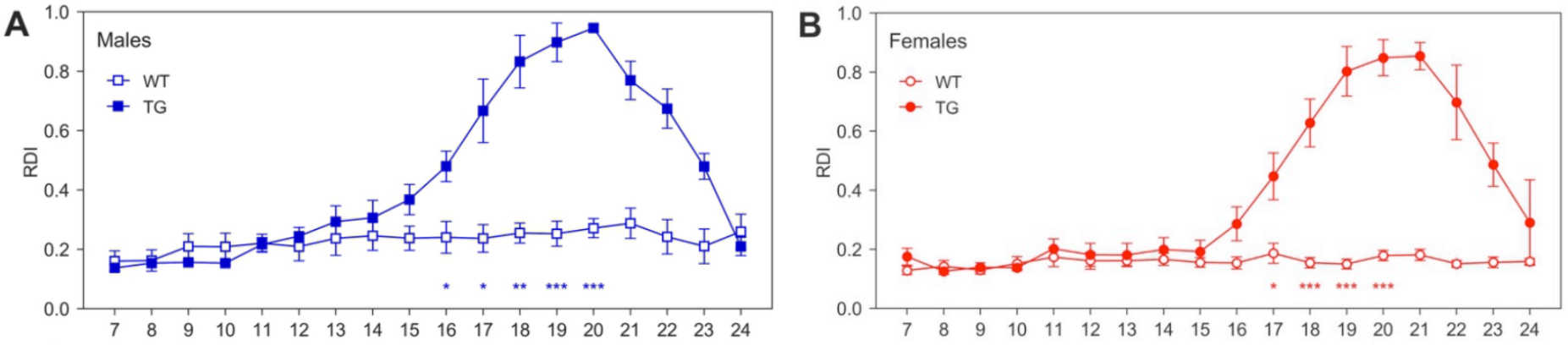
Quantitative assessment of activity: **Rest Disturbance Index (RDI)**

Sample Entropy: Assess variation with interrelated data series

→

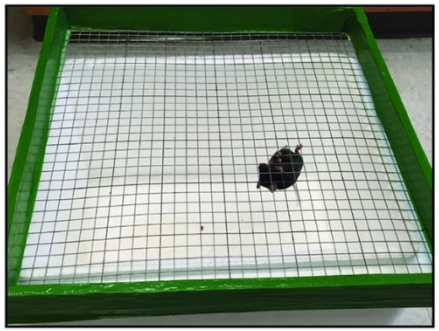
Physiological time series (e.g., ECG) to assess cardiac issues

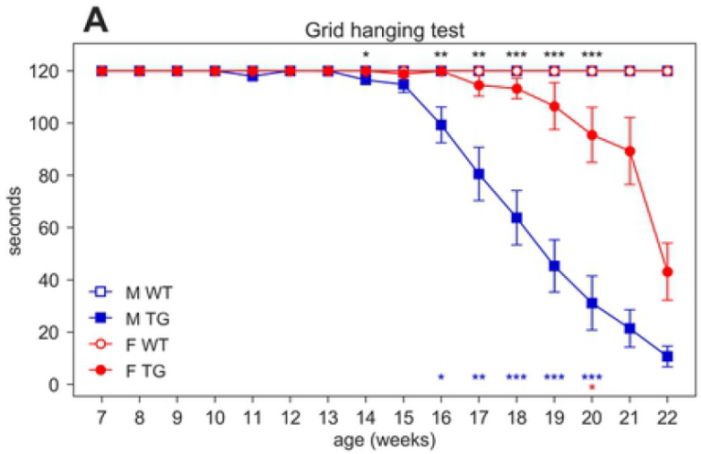
Activity

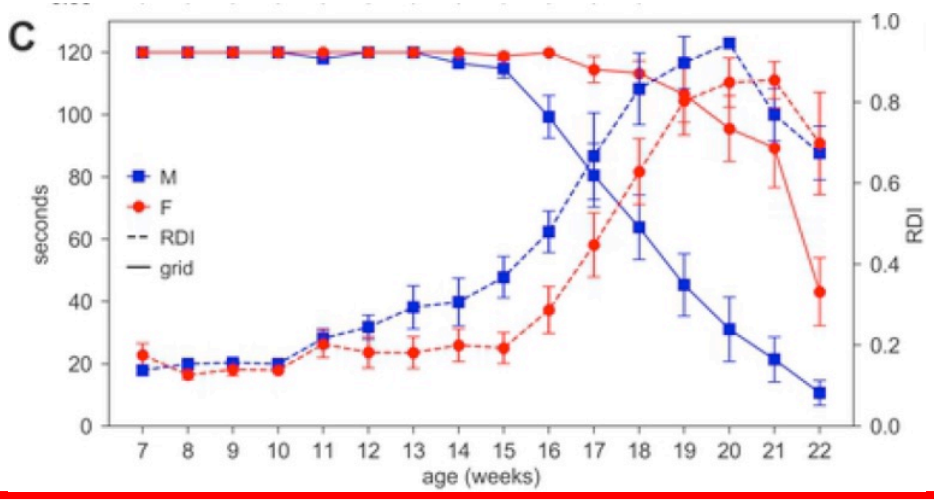


Study example # 2:
ALS progression in transgenic SOD mice

Quantitative assessment: **Rest Disturbance Index (RDI) vs. Grid Test**







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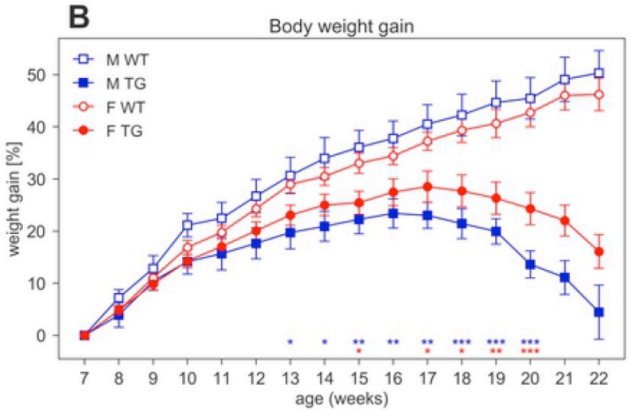
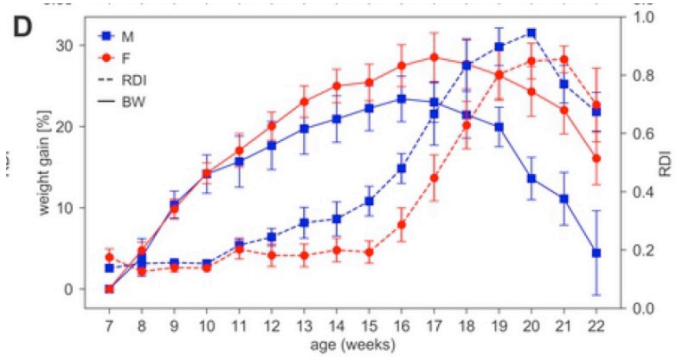
Study example # 2:
ALS progression in transgenic SOD mice

Quantitative assessment: **Rest Disturbance Index (RDI) vs. Body Weight**





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Study example # 2: ALS progression in transgenic SOD mice

Study conclusions:

- Proven that RDI can be used as biomarkers for early ALS identification
- Gender differences in the time development of the disease in all parameters

DIGILAB website

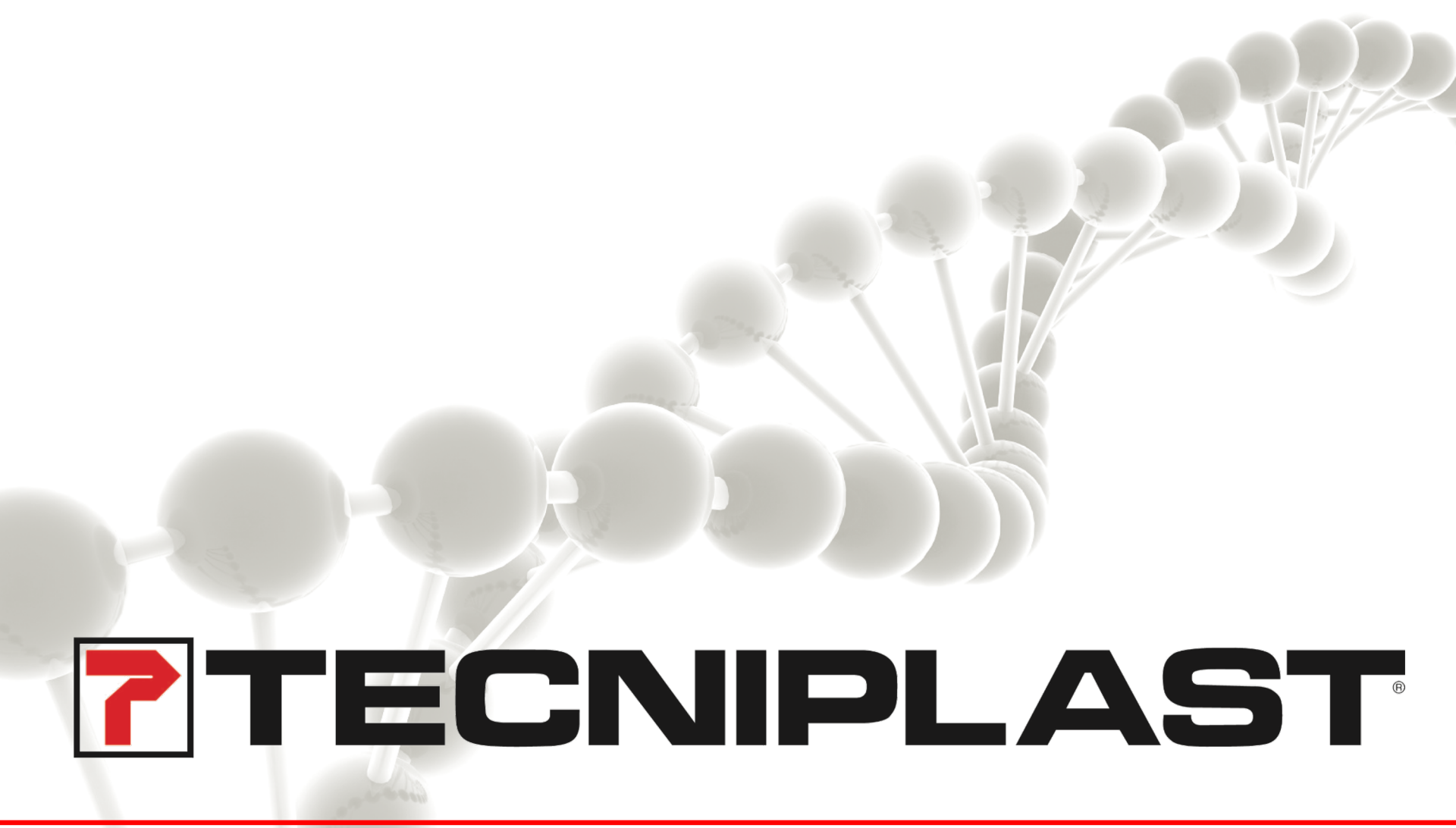
For latest peer-reviewed studies (and any other informative material), please visit:

https://digitalcage-tecniplast.com/en/dvc-scientific-papers.html

DVC® Overall Conclusions

Lack of study reproducibility, unexpected outcomes from experiment, poor translational results, are possibly linked to a similar issue:
lack of full knowledge and understanding of all the events occurring in an Animal Facility.

We strongly believe that the **DVC® is a revolutionary opportunity to fill this lack and provide to final users** (Facility people and/or Researchers) the possibility to interpret, analyze and generate results that can support and enhance science.



TECNIPLAST®

Thank You!