High-quality Simultaneous Measurements of Rodent Behaviour, Tracking, Physiology, Sleep Stages and Ultrasounds

a report by
Metris B.V.

Metris B.V. offers and supports highly advanced non-invasive laboratory equipment for *in vivo* research with freely moving rodents. The systems are modular, complementary and ready to be coupled to other systems:

- LABORAS is innovative equipment for the automated assessment of behaviours and position tracking based on vibration analysis;
- SonoTrack records and analyses full-spectrum ultrasonic vocalisations; and
- SleepSign is software to determine sleep stages from electroencephalogram (EEG) and electromyogram (EMG) data.

The latest development is the integration of LABORAS with the Datasciences Telemetry system. Validation of the integration published in August 2005 shows that integrated solutions of the animal research laboratories is improving the quality of research while decreasing time, money and number of experiments and animals.

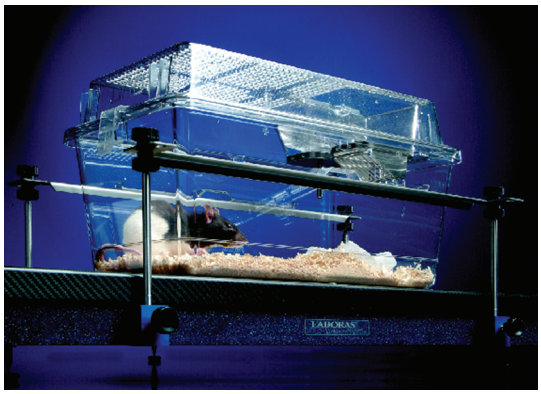
LABORAS Automates Behavioural Scoring and Tracking

LABORAS is an efficient and validated non-invasive technology, based on vibration measurement and pattern recognition techniques.[1–7] The triangular shaped sensor platform (see *Figure 1*) records all movements evoked by the animal. Each behaviour has its own unique signature of vibration characteristics, which can be detected by the LABORAS software to identify a behaviour. The system is currently the only validated equipment on the market that is able to determine a large number of different behaviours without a human observer.

LABORAS can detect a wide range of different behaviours such as climbing, drinking, eating, grooming, immobility, locomotion and rearing. In addition it provides tracking parameters – position, speed, maximum speed, average speed, travelled distance and position distribution.

Metris continuously offers new validated behaviour detection software to detect both normal and drug-induced behaviours such as hind-limb licking detection, scratching behaviour and circling behaviour. The unique functionality enables the researcher to perform behavioural research faster, more consistently and more efficiently than that which is possible with human observation or other technologies.

Figure 1: The LABORAS System



LABORAS – Let the Computer Score

There are several advantages of using the LABORAS system.

For Management

- Increases efficiency of research.
- 'One stop shop' for rodent tracking and automated behaviour identification.
- Reduces experimental lead time, animals and costs.
- GLP-compliance (21CFR/Part 11).
- Speeds up experimental throughput.
- Combines quality and efficiency.
- Efficient use of equipment.
- Replaces several dedicated systems.

Table 1: Examples of Applications of LABORAS in Drug Discovery

Test	Result
Behavioural phenotyping transgenic mice[8–10] development	Target discovery for future drug
Primary observation test	Adverse drug effects
Open field test[10]	E.g. sedative, stimulant, anxiolytic, anxiogenic properties
Pain test (formaline induced hindlimb licking)[11]	Analgesic properties
Telemetry and behaviour[17]	Integrated cardiovascular, CNS and behavioural data
Dermatology test (scratching)	Skin protective effects
Effect on pharmacologically-induced effects, such as: • m-CPP hypolocomotion and hypophagia;[6] • 8-OH-DPAT hyperlocomotion;[6] • d-Amphetamine hyperactivity;[6] • Angiotensin II polydypsia;[6] • Orexin-A induced grooming;[7] • excessive climbing; and • turning/circling behaviour.	Pharmacological efficacy of (potential) leads
Near Future Applications	
Antagonism of pharmacologically induced effects, such as • tremors; • seizures; • wet dog shakes; and • startle response	Pharmacological antagonist efficacy, e.g. anticonvulsant activity
Future Applications	
Respiration	Adverse effects
Heart beat	Efficacy and safety issues
Sniffing Digging Jumping	Exploration/anxiety measure
Energy spent	Metabolic effects
Disturbed feeding/drinking patterns (Eating/drinking)	Hypo- or hyperphagia; Hypo- or polydipsia

- Flexible update and extension options.

For Researchers

- Objectivity and standardisation of data collection.
- Reduces inter- and intra-observer bias.
- Easy to use and matches specific research needs (as it was designed for and with the help of researchers).
- Measurement in total darkness possible (no video cameras or light required).
- Limited amount of data (raw data can be kept, no large video data files).
- Home cage type environment.
- Standard data output (time-tagged files).
- Result summaries over user-definable windows (exportable to Excel and statistical packages).

LABORAS enables the researcher to carry out more and increasingly effective experiments in less time using fewer animals and less equipment.

LABORAS in Drug Discovery

Target Discovery

Behavioural phenotyping of transgenic animals is a pivotal element in chasing promising targets for future drug development (FDD). To screen huge amounts of genetic modifications, a quick and easy behavioural method with high quality and capacity, such as LABORAS, is the answer.[8–10]

(Safety) Pharmacology and Toxicology

When studying the efficacy and safety of a future drug, animal behaviour is a very important and sensitive end-point. A standardised high-capacity behavioural screening method increases efficiency and quality of results, and reduces lead-times. When animals have telemetry transmitters for cardiovascular (CV) or central nervous system (CNS) measurements, they can simultaneously be put on a LABORAS sensor platform to assess concurrent behaviours and tracking. Data of both systems can be completely integrated and processed (see *Integration of LABORAS and Telemetry* sub-heading).

Pain Research

LABORAS has a specially developed algorithm dedicated to pain research. After administration of the painful fluid the hind-limb licking response is measured to investigate the effect of pain reducing drugs.

Allergy Research and Dermatology

LABORAS offers the automatic detection of scratching behaviour for mice and rats.

CNS Research

The system can be used to investigate pharmacological properties of drugs, e.g. agonism/antagonism of m-chlorophenylpiperazine (m-CPP) induced hypolocomotion, 8-Hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) induced hyperlocomotion, d-amphetamine induced hyperactivity, angiotensin II induced polydipsia or orexin-A induced excessive grooming.[6] LABORAS is currently used as an open field test measuring locomotion, immobility and position. LABORAS also includes special software for circling behaviour analysis.

LABORAS data is reliable and free of inter- and intra-observer bias or anticipated scoring, thereby providing a standard behavioural measurement worldwide.

LABORAS Compared with Other Research Equipment

The identification of behavioural elements with LABORAS is far more sophisticated and extensive than with video systems or 'infrared beam breaking' systems. Due to the fact that LABORAS measures vibrations, it does not rely on (infrared) light or multiple (special) cameras and does not have the problems of reflections, poor resolution or large data files. Compared with infrared beam breaking systems LABORAS provides a significantly higher spatial resolution.

Conclusions

LABORAS enables the researcher to carry out more behavioural experiments in a shorter time with fewer animals and less equipment, while obtaining higher quality data. The LABORAS system can be a valuable tool to standardise behavioural measurements, particularly for disciplines falling under good laboratory practice (GLP) regulations.

SonoTrack for Recording and Analysis of Ultrasounds

SonoTrack is the first full spectrum ultrasound recording and analysis system on the market designed for use in animal research laboratories. SonoTrack is an easy to use, non-invasive measurement system (see *Figure 2*). It opens possibilities to measure pain, distress, anxiety, comfort, social interaction and general animal welfare based on ultrasonic vocalisations.

Ultrasonic Vocalisations

Rats and mice produce ultrasonic vocalisations in a variety of situations, for instance in response to stress, anxiety and pain (22kHz),[12,13] or during social interaction such as sexual behaviour (50kHz).[14] Small rat pups emit ultrasounds in response to separation from their mother and littermates (35kHz).[15,16] These ultrasonic vocalisations can be used as an indicator of emotional and motivational status. In animal models of stress, anxiety, pain or sexual behaviour, but also in studies of the wellbeing of animals, ultrasound is an accepted and sensitive parameter.

SonoTrack

SonoTrack is a new and non-invasive method that does not involve bat detectors. By using highly

Figure 2: The SonoTrack System

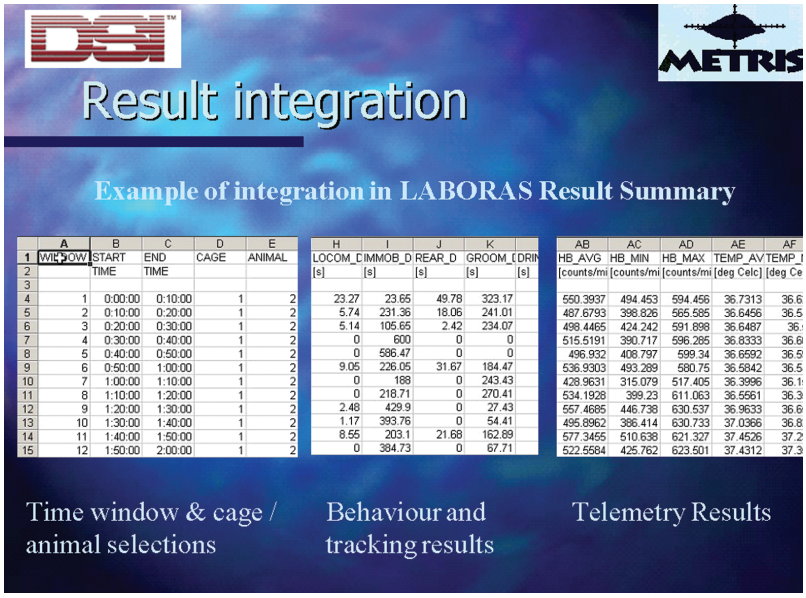
sensitive microphones and sophisticated electronics, SonoTrack is able to receive ultrasonic vocalisations within the full frequency spectrum of 15kHz to 100kHz. The ultrasounds can be visualised in various types of graphical presentations – they can be transformed into audible signals for humans and they can be investigated and counted in user-definable frequency bands. The final experiment results are presented in easily exportable file formats. The resulting data include the number of calls, time of call, call frequency and call duration.

SonoTrack – A Sound Idea

The advantages of SonoTrack include:

- full spectrum scanning, recording and analysis without the need of pre-tuning;
- state-of-the-art electronics and data-acquisition technology for the best signal to noise ratio;
- extensive possibilities for elimination of background noise;
- sensitive for low level calls;
- smart microphone set-up for multi-cage experiments without the necessity of soundproof chambers;
- multi-channel continuous recording (up to four independent channels);
- functions for extensive analysis of ultrasound calls;
- graphical presentations (frequency graphs, two-dimensional (2-D) and 3-D sonograms);
- transformation of ultrasounds into audible sounds;
- export of recordings to commonly used audio file formats, such as .wav files;
- smart data storage to prevent unnecessary use of hard disk space;

Figure 3: Example of the Integration LABORAS and TELEMETRY – Telemetry Data Imported in LABORAS Result Summary



- quantification of calls in user-definable frequency bands (time-tagged files); and
- easy export of the results to other software packages.

Conclusions

With SonoTrack it is easy to determine the nature of the ultrasonic vocalisations produced by an animal. Hereby, ultrasonic range vocalisations (USV) will become a new reliable parameter for behaviour analysis and animal welfare.

SleepSign for Automatic Sleep Stage Analysis

SleepSign for Animal is the latest product in the Metris portfolio. Metris distributes this product in Europe on behalf of Kissei Comtech America. It enables further expansion of automated animal behaviour research. SleepSign for Animal is a software package that can distinguish several sleep stages (e.g. rapid eye movement (REM), non-rapid eye movement (NREM) and wake) in freely moving animals using EEG and EMG data obtained by telemetry.

SleepSign for Animal is a strong tool that proved itself in sleep analysis, neurology and pharmacological research. It offers the ability for both in-depth analysis of sleep stages and easy automated sleep stages detection. It drastically reduces the time and labour spent on sleep stage scoring and enables you to generate standard types of reports with only a few keystrokes.

SleepSign uses EEG and EMG data in many possible data formats. The possibility of using EEG and EMG data from the Datasciences Telemetry system enables integration with LABORAS. This development will be offered after validation by an independent research organisation. Metris strives to integrate measurement systems in order to provide laboratories with a total animal behaviour analysis solution – day- and night-time simultaneous recordings of behavioural, physiological and acoustical data. It will increase both the flexibility and capacity of test facilities.

SleepSign – The Vigilant Sleep Monitor

The advantages of SleepSign include:

- both in-depth analysis of sleep stages and automatic sleep stages detection;
- an easy to use package;
- possibility of user-definable sleep stages;
- analysis, processing or editing of bioelectric signals (EEG and EMG);
- combination with behaviour analysis provided by LABORAS;
- combination with telemetry data; and
- combination with ultrasound recording.

Integration of LABORAS and Telemetry

Metris co-operates with several alliance partners to work towards a 'total solution' in automated behaviour analysis, which will help to gain the most out of a single experiment. We therefore give high priority to the development of the integration of methods.

The integration of LABORAS and the Datasciences Telemetry system enables the researcher to obtain the highly accurate behaviour analysis provided by LABORAS in combination with ECG, EEG, EMG, temperature and blood pressure data of the telemetry implants, all in one experiment.[17,18]

The parallel data acquisition greatly enhances the efficiency and quality of animal experiments. It reduces the number of experiments, it saves animals, time and money and makes efficient use of equipment compared with serial testing. It eliminates external variation and thereby enhances the power of experiments. It helps to establish causal relationships in datasets by direct time-matched coupling of several parameters – from different biological systems – in one single animal. This leads to a better interpretation of biological processes than using separate data.

LABORAS and TELEMETRY – 1 + 1 = 3!

The are many advantages of integrating LABORAS with Telemetry.

For Management

- Increases efficiency of research;
  - increases the information from a single animal;
  - reduces the number of separate or serial experiments;
  - increases overall research capacity (throughput);
  - Saves animals, time and money.

- Efficient use of equipment;
  - replaces several dedicated systems;
  - flexible extension options;
  - software of both systems runs on one PC at the same time; and
  - no hardware changes to existing systems.

- Next step towards integration with more systems.

For Researchers

- Better understanding of biological processes;
  - establishing causal relationships.

- New insights in area of phenotyping transgenic animals;
  - measures more independent parameters;
  - measures various biological systems simultaneously; and

- Increased quality of data by simultaneous testing;
  - parameters like activity (DSI) and locomotion (LABORAS) can be used to check data quality by cross checking the data of both systems.

Combining behaviour and physiology experiments makes sense. Why waste valuable information any longer?

Future Research Options

Ultimately, behaviour, tracking, physiology, sleep stages and ultrasounds can all be measured and analysed simultaneously in one experiment. ■

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