

High-throughput and Automated Behavioural Screening of Normal and Genetically Modified Mice

a report by

Hans-Peter Lipp

Chief Scientific Officer of NewBehavior, Professor of Neuroanatomy & Behaviour at the University of Zürich, Head of Competence Centre for Advanced Behavioural Assessment of Rodents

Reasons for Behavioural Testing

Traditionally, behavioural analysis of laboratory animals has been an established tool in research and testing of drugs targeting the nervous system. However, every circulating drug exerts indirect effects on the central nervous system (CNS), chiefly by means of hormonal communication or feedback from the peripheral and enteric nervous system. While the exact mechanisms of communication are often poorly understood and thus not suitable for the analysis of causal mechanisms, indirect behavioural effects are a common observation. For example, any gross malaise, or cure thereof, results in changes of spontaneous activity. More subtle effects do not translate in overt behavioural changes, but affect motivation, decision-making and cognitive performance, as is clearly seen in humans. However, recognising such systemic effects in mice requires careful observation, lengthy testing and profound methodological know-how. For reasons explained below, this made thorough behavioural screening for systemic drug effects impossible until now.

At Which Stage of the Modern Drug Discovery Process is Behavioural Analysis Useful?

Drug discovery and development commonly include target identification, lead identification, lead optimisation, pre-clinical studies and clinical trials. Identification focuses on measurable interactions between compounds and target, being increasingly accomplished by primary high-throughput screening (HTS) using automated detection platforms and liquid-handling technologies.¹ These more recent approaches generate a high number of candidate substances. Secondary and tertiary HTS screens are then needed for lead identification and optimisation, determining if a target is critically involved in a disease process. However, the high number of candidate compounds already presents a formidable challenge as it requires adaptation of techniques from functional genomics and proteomics, including cell-based assays. Evidently, the immense number of generated compounds makes it

highly desirable to eliminate (or flag) primarily every substance with potential systemic side effects, while systemic gain-of-function properties might be tested at a later stage. Behavioural HTS would be a biological relevant procedure for such an early triage, but has not been realised thus far.

Current Barriers for Implementing Behavioural and Cognitive HTS

Behavioural analysis of mice, including cognitive components, has become an accepted tool for behavioural neuroscience and pharmacology of the CNS. Traditionally, animals are subjected to a battery of tests designed for demonstrating changes in spatial or fear-related memory, motivation, exploration or species-specific abilities thought to reflect the operation of specific brain systems. In addition, a number of tests have been designed to model processes observed in human diseases of the CNS. Thus, every laboratory specialising in behavioural phenotyping is forced to maintain a collection of more or less popular test set-ups thought to assess various functions or malfunctions of the CNS. This situation does not meet the demands of drug discovery, for the following reasons experienced by behavioural screening of several thousands of genetically modified mice.

- In order to discover unpredicted behavioural changes signalling a modification of CNS function, animals must undergo a battery of tests challenging functionality of different brain systems. Even for relatively small numbers of individual test subjects, this requires ample space and considerable man-power. Practical experience shows that a classic preliminary behavioural screen of about 100 mice requires between four and six different tests, and three man-months; a larger screen using more tests up to six man-months, even when using highly standardised procedures and automated data analysis. Moreover, conducting, analysing and interpreting test results needs highly specialised

1. Hertzberg R P, Pope A J, "High-throughput Screening: NewTechnology for the 21st century", *Curr. Opin. Chem. Biol.* (2000), 4 (4), pp. 445–451.

practical skills of operators and profound theoretical knowledge and long-term experience of scientists. Thus, the main obstacles for behavioural HTS are costs in terms of space, salaries and professional education on the job.

- Behavioural research has proven to be remarkably resistant to cross-laboratory standardisation. One reason is that existing behavioural tests can be modified infinitely, and novel tests easily invented. On the other hand, testing laboratories are reluctant to adopt novel tests and tend to stick to their own standardised procedures. However, recent research has also found substantial cross-laboratory variations of scores in manually conducted tests.² This has led to the recognition of human handling as a substantial confounding factor.^{3,4} However, potentially critical human handling factors such as experience, nervousness or even body smell are practically impossible to standardise.
- Behavioural mass screening of mice is a novel methodological and conceptual challenge for behavioural science traditionally focussing on psychological or neuropsychological concepts of brain function and malfunction. Academic and industrial expertise is largely lacking.

Recent Attempts of Behavioural HTS

The need for behavioural HTS in mice finds its roots, historically, in the explosion of genetically modified mouse lines and the N-ethyl-N-nitrosourea (ENU) mutagenesis programmes that have replaced rats as the main laboratory animal for behavioural testing. Consequently, drug discovery is shifting to mice as well, profiting from the extensive knowledge of the mouse genome. This trend towards HTS has been recognised by research laboratories and the traditional industry supplying equipment for behavioural analysis. Different strategies are being followed. One (prevailing) strategy is multiplication of existing conventional test devices (e.g. operant boxes, shock chambers, metabolic cages) for single animals, multiple boxes being controlled by computers. While technically the most easy way, this ‘inflationary’ approach requires multiplication of each specialised test system, does not address the main costs of HTS (space, personnel, education) and neglects the issue of

optimised analysis of huge data sets, relegating this problem to the user because the manufacturers lack, thus far, the practical know-how for HTS.

Another approach is home cage testing, eliminating the need for laboratory space and handling by humans. It has been mainly implemented as activity scoring of individually caged animals and has been proven useful in chronobiology and monitoring of slowly progressing diseases.⁵ At present, the merit of this simple approach is economical screening for gross malfunctions of the CNS; its disadvantage being the need to keep isolated mice over long periods, a practice that, in the near future, is likely to meet legislative restriction due to animal welfare issues. More recently, some manufacturers have attempted to place or attach some of their devices to small home cages containing individual mice, or construct special home cages permitting video-tracking of single animals by means of established tracking systems. While allowing for prolonged testing under relatively stress-free conditions, these strategies are clearly multiplicative in terms of financial investment.

To the author’s knowledge, NewBehavior is the only company offering a second-generation system that has been specifically designed to cope with the requirements of cost-effective, compact and automated behavioural HTS including drug discovery.

Design Features of INTELLICAGE

The design of INTELLICAGE incorporates knowledge from three lines of research: 30 years of experience in neurobehavioural genetics of existing or selectively-bred mouse lines, 15 years of behavioural screening of genetically modified mice, and, rather uniquely, 12 years of experience in analysing behaviour and learning of mice living in social groups in large naturalistic outdoor pens.^{6,7} The system had to meet a set of criteria as shown in *Table 1*.

Realisation and Operation of the INTELLICAGE System

Figure 1 shows a single INTELLICAGE of the second-generation, based on working four years with (functionally identical) prototype systems. Each cage houses between eight and 16 mice carrying

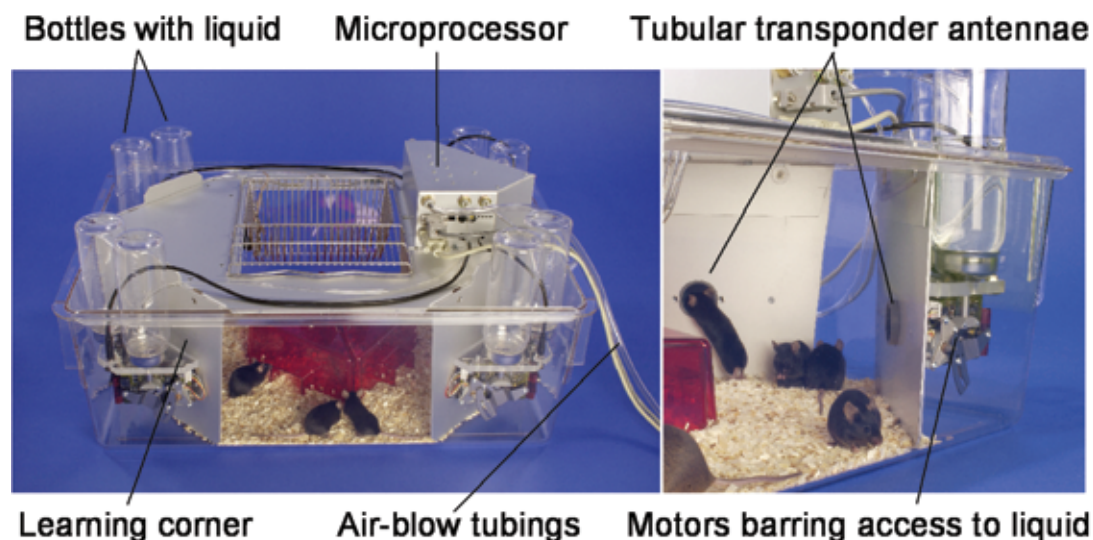
2. Crabbe J C et al., “Genetics of Mouse Behavior: Interactions with Laboratory Environment”, *Science* (1999), 284, pp. 1,670–1,672.

3. Chesler E J et al., “Influences of Laboratory Environment on Behavior”, *Nat Neurosci* (2002), 5 (11), pp. 1,101–1,102.

4. Würbel H, “Behavioral Phenotyping Enhanced – Beyond (environmental) Standardization”, *Genes, Brain and Behavior* (2002), 1 (1), pp. 3–8.

5. Dell’Omo G et al., “Automated Home Cage Monitoring of Mice Infected with BSE and Scrapie Differentiates Early Behavioural Changes According to Prion Strain”, *European Journal of Neuroscience* (2002), 16, pp. 735–742.

Figure 1: INTELLICAGE System Housing Mice Undergoing Automatically Permanent Monitoring and Training for Various Learning Tasks Designed to Challenge Different Brain Systems. Thus, any Malfunction Due to CNS Malfunction, or Somatic Effects of Drugs or Drug Candidates Inducing Malaise, can be Discovered Within a Few Days Maximally



commercially available microchips for identification (TROVAN and DATAMARS). One controller computer can handle up to eight cages, monitoring or testing a maximum of 128 mice, each one individually, multitasking for different tests possible. The flow of information between computers and test cages is shown in *Figure 2*.

Essentially, an INTELLICAGE is a behavioural micro-laboratory permitting automated individual assessment of spontaneous and cognitive behaviour typical for mice (see *Table 2*). Each cage contains four identical test corners accommodating one mouse at a time. A mouse entering a test corner is identified by passive transponders implanted subcutaneously. Depending on schedule, the computer can reward correct responses by opening access to the liquid bottles, or it may refuse access or even punish incorrect choices by delivering air-puffs. Spontaneous behaviour such as basic activity levels, circadian variations, exploratory behaviour, neophobia and behavioural stereotypies are concomitantly assessed in most testing modules. Social hierarchies and changes thereof can be assessed by competition for reward after programmable deprivation periods. Taken together, the INTELLICAGE system represents an almost universal tool for fully automated behavioural HTS relegated to animal stables yet allows, for expert users, sophisticated behavioural analysis for mechanistic purposes.

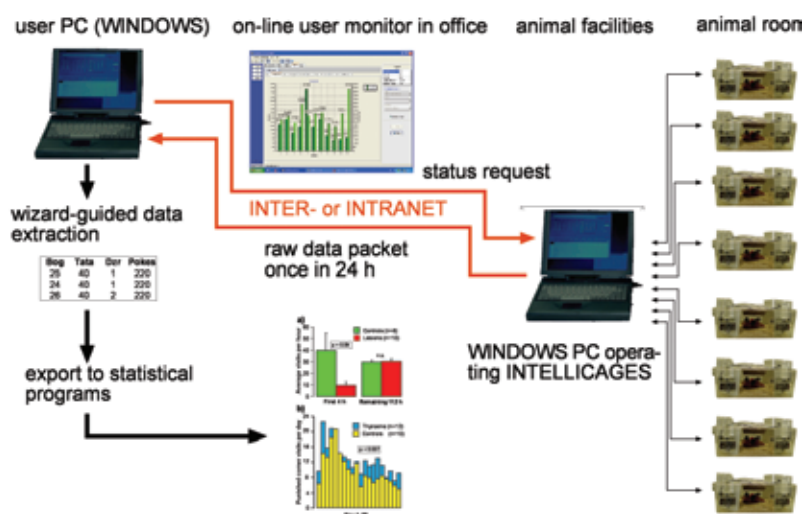
Table 1: Design Criteria

Behavioural criteria
Automated assessment of spontaneous and cognitive behaviour of <i>individual</i> mice living in social groups, with minimal handling by humans
Opportunity to keep control and experimental animals in the same group
Execution of widely used behavioural tests checking various brain systems (<i>Table 2</i>)
Permitting controlled uptake of drugs and gustatory discrimination learning
Minimizing measures unfamiliar to the behavioural community
Allowing both standardised behavioural HTS and low-throughput mechanistic analysis
Practicality and economy
Multicage systems suitable for operating inside mouse facilities fitting commercially available animal racks, saving expensive lab space for other purposes
Placement in commercially available rodent cages (Techniplast 2000 with filter covers) large enough for housing several mice and permitting analysis of spatial learning
Easy change of cages for weekly cleaning
Minimal supervision without special professional training
Remote graphic monitoring of test progress from the office or through internet
Flexibility of use
Offering adaptation to customer-specific demands by software rather than hardware
Training mice and monitoring of up to 8 cages with a maximum of 128 animals
Multitasking permitting to run simultaneously different tests, within and between cages
Storing the complete sequence of events for off-line analysis
Preventing loss of data by interrupts of electrical power
Pausing of ongoing experiments
Software packages including pre-programmed test modules and extraction of relevant data for specific customer groups
Wizard-guided export of relevant tabulated data to statistical programs
Individual schedule programming and data extraction for experts

6. Vyssotski A L et al., "Long-term Monitoring of Hippocampus-Dependent Behavior in Naturalistic Settings: Mutant Mice Lacking the Neurotrophin Receptor TrkB in the Forebrain Show Spatial Learning but Impaired Behavioral Flexibility", *Hippocampus* (2002), 12, pp. 27–38.
7. Dell'Omo G et al., "An Automated System Based on Microchips for Monitoring Individual Activity of Wild Small Mammals", *J. Exp. Zoology* (1998), 280, pp. 97–99.

Table 2: Examples of Programmable Behavioural Tests

Spontaneous behaviour
Basic activity levels, circadian activity
Anxiety, neophobia and exploration
Spatial and temporal
Stereotypical place preferences
Spatial preference and avoidance learning
Spatial reversal learning
Spontaneous alternation
Temporal conditioning
Temporo-spatial conditioning
Systematic patrolling schedules
Radial maze-like patrolling
Discrimination learning & preferences
Visual discrimination
Gustatory discrimination learning
Spontaneous drug preference or avoidance
Memory
Procedural memory
Habituation
Spatial short-term (working memory)
Visceral/gustatory memory
Social & others
Competition rank orders
Approach-avoidance conflicts
Operant conditioning
Procedural learning
Fixed ratio conditioning (motivation)
DRL (Differential reinforcement of low responding, response inhibition, timing)

Figure 2: While the System (including up to eight cages run by one small computer) is Located in the Animal Facilities, Remote Occasional Supervision from the Office Via Intranet is all that is Needed

Data are sent to the office computer in regular intervals and analysed there by means of extraction programs tailored to extract functionally relevant information.

For methodological, financial and species-specific reasons, the system does not use solid food for reward, the only planned add-on being a feeder system measuring individual food consumption. Movements across the cage and contacts between particular mice cannot be recorded by means of transponder technology. Finally, INTELLICAGE operates most efficiently over long periods with female mice; while males will sooner or later require the insertion of compartment barriers housing two to

three males. However, this limits merely the analysis of spatial behaviour. Contrary to a widely held belief, behavioural analysis is not confounded by social interactions, and it has only been shown recently that standardisation of behavioural testing in mice is not prevented by enriched environment.⁸

It should be emphasised that mice need not be kept permanently in INTELLICAGES. Test cohorts can be formed and kept in ordinary cages. Typical test programmes last one week. Normally, the animals adapt very rapidly when being re-introduced to the system. This allows for efficient long-term monitoring. On the other hand, INTELLICAGE has not been designed to replace conventional behavioural laboratories. It simply relegates the chore of monotonous manual testing to screening robots, freeing expertise of employees for detailed analysis of behavioural problems flagged by HTS.

How to Use INTELLICAGE Systems in Drug Discovery

Two extreme examples obtained at the University of Zürich illustrate the power of the system to rapidly identify severe problems or, conversely, to discover subtle behavioural changes by working over long periods. *Figure 3a* shows how INTELLICAGE recognises, within hours, hippocampally lesioned mice kept with controls in the same cage. *Figure 3b* shows how the system ran a particular testing schedule over 20 days and eventually recognised a very subtle toxicological effect. The point is that the treatment, a postnatal hyperthyroidism, had been applied to the great-grandfathers of the mice tested. By unknown mechanisms, the behavioural alterations had been paternally transmitted to the following generations, being evidenced by conventional behavioural testing in the first and second generation but fading in the third one in which only INTELLICAGE could detect remnant subtle impairments.

Such prolonged search for suspected effects is not required for behavioural HTS. There are substantial reasons to believe that INTELLICAGE will permit a relatively simple and automated procedure for HTS of compounds that identifies potentially negative side effects already at an early stage of drug discovery and development, permitting lead validation by triage of candidate drugs. As behavioural HTS is based one third on technology but two-thirds on experience and behavioural know-how, the procedure cannot be published here for reasons of intellectual and commercial priority. Briefly, the screen includes a cross-section of behavioural parameters found in about 60 mutant mouse lines with behavioural

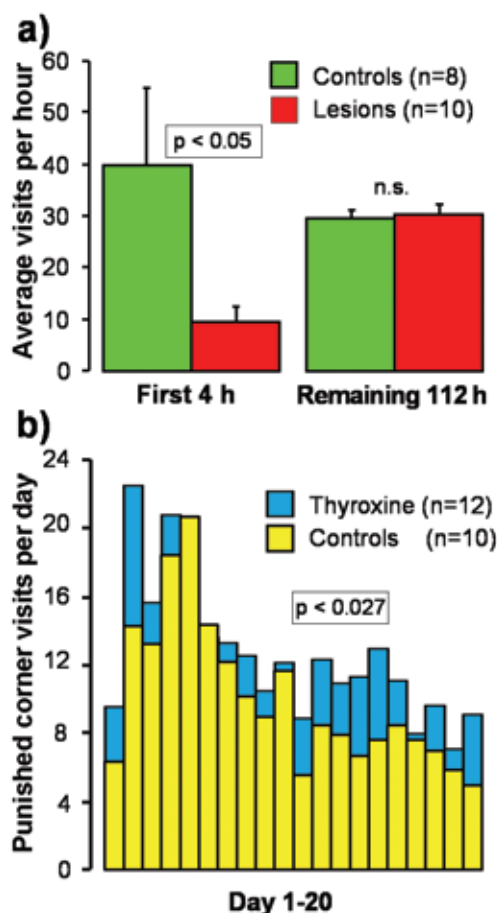
8. Wolfer D P et al., "Cage enrichment and mouse behaviour", *Nature* (2004), 32, Dec 16, pp. 821-822.

phenotypes originating from specific or non-specific malfunctions of the CNS. The other part of the screen is based on the opportunity to deliver water-soluble and orally ingested test compounds (probably the most salient feature of any candidate drug), and to profit from the extremely finely tuned neural systems in the mouse brain registering and memorising somatic physiological disturbances. NewBehavior is willing to cooperate individually with interested partners in adapting the INTELLICAGE system to specific needs in drug discovery and development.

Outlook

Will behavioural HTS in this or other forms ever be employed in drug discovery and development? After all, the sheer number of existing or future compounds is immense, and even compact and economical systems such as INTELLICAGE will require considerable investment. Because the units for such bio-assays are individual organisms, an optimal use will require careful scientific and economic consideration including choice of optimal genetic backgrounds. On the other hand, the early detection of negative systemic side-effects, or, conversely, possible addictive and beneficial effects, are likely to give a higher economic yield in the process of drug discovery because it reduces the risk of ending an advanced lead by the standard toxicological testing of animals.⁹ The second reason for being affirmative with regards to implementation of behavioural HTS is that it will be requested by law sooner or later. The technical capabilities and know-how to accomplish such screens exists, it is commercially available, and can be adapted to the demands of research and industry. ■

Figure 3: Rapid Discovery of Major Behavioural Problems and Protracted Search for Suspected Subtle Problems



a) Mice with large lesions of the hippocampus hesitate to enter the test chambers during the first four hours of placement in INTELLICAGE because of neophobia. The remaining observation period showed that the lesioned mice eventually adapted to the new situation. b) Mice that did not show any behavioural deficits in manual classic testing were subjected to a 20-day testing procedure. Animals were assigned to one corner where they could obtain water, while visits of the other corners were punished by air-blows. The group of mice showing a continually higher rate of entries into wrong compartments included the descendents of grand-grandfathers who had received a subliminally toxic treatment as pups. Neither the way of paternal germ-line transmission nor the functional significance of the behavioural change is known, but the system was able to discover toxicological effects originating from a distant past.

Contact Information

NewBehavior AG
 Hardturmstrasse 76
 CH-8005 Zürich
 Switzerland
 Telefon: +411 272 7344
 Fax: +411 440 0381
 e-Mail: info@newbehavior.com
<http://www.newbehavior.com>

9. Li A P, "An Integrated, Multidisciplinary Approach for Drug Safety Assessment", *Drug Discov Today* (2004), 9 (16), pp. 687–693.