# The PhenoTyper automated home cage environment as a high throughput tool to detect behavioral abnormalities in mutant mice

M. Loos
*Center for Neurogenomics and Cognitive Research, VU University, Amsterdam, The Netherlands, maarten.loos@falw.vu.nl*
*The Neuro-Bsik Mouse Phenomics consortium*[1]

Over the last decades, the high throughput toolbox of neurobiologists to measure molecular changes in the brain has grown rapidly. The molecular effects of induced genetic mutations or pharmacological treatment relevant to psychiatric disorders can now be studied on a large scale. In contrast, to investigate the behavioral consequences of genetic mutations or pharmacological treatment, researchers rely on multiple stand-alone tests, each of which taxes a specific aspect of a different behavioral domain. It is clear that these conventional stand-alone tests give highly valuable detailed information about specific psychological constructs. However, for the purpose of phenotyping larger numbers of novel mutants or novel pharmaceuticals, performing a battery of these conventional tests has two major drawbacks. First of all, performing a battery of tests requires a substantial amount of time and resources. Secondly, it has proven to be difficult to reproducibly measure behavioral effects in different laboratories, especially subtle ones [1]. This difficulty in reproducibility may partly be due to a multitude of human-animal interactions required for these behavioral tests. Therefore, automation may be a way of reducing the required amount of time and resources involved with behavioral phenotyping. In addition, automation will circumvent human-animal interactions, and thereby holds the promise to increase reproducibility of behavioral results across laboratories. The development of automated phenotyping strategies has only started recently, for instance using automated home cage observations[2]. More research is needed, especially to increase throughput and to investigate the sensitivity and reproducibility of these automated behavioral tests.

Within the framework of the Dutch Neuro-Bsik Mouse Phenomics project, an automated high throughput screening protocol was developed, consisting of an automated home cage environment (PhenoTyper®) and separate home cages equipped with running wheels. Neuro-Bsik Mouse Phenomics is a Dutch consortium of 11 academic research groups and two companies. The consortium aims to contribute to the understanding of brain disorders by developing novel mouse models for brain disorders. These models will be initially identified using the automated high throughput screening protocol to analyze mouse behavior and subsequently be analyzed in depth at different levels of complexity using the specific expertise of each of the academic partners.

For the aims of the consortium, it is essential to investigate whether the developed automated high throughput screening protocol can discriminate mouse behavior with sufficient resolution, e.g. mutant from wild type mice or specific behavioral differences between common inbred lines of mice. Secondly, if differences in behavior between genotypes are detected, the high throughput screen should indicate in which behavioral domain (e.g. circadian rhythm, anxiety, learning) further in-depth analysis might be fruitful.

To this end, the behavior of around fifty genetically diverse mouse lines is currently being measured using the automated high throughput screening protocol comprising engineered mutants, eight common inbred strains and the panel of BXD recombinant inbred strains. After acclimatization to the facility for at least one week, each mouse is individually housed in one of the forty-eight PhenoTyper cages for six and a half days. Using newly developed soft- and hard-ware, i.e. a video camera, Lick-O-meters and visual and acoustical stimuli, numerous measures of spontaneous behavior are recorded throughout these six days. During the last three dark phases, mice are exposed to three tests tax aspects of cognition and anxiety; an instrumental conditioning task using a device that dispenses palatable food, an avoidance learning task using illumination in the shelter, and an anxiety test using a bright spot of light on the cage floor. To automate these tasks, a series of new software applications have been developed for high-throughput data acquisition, data processing and statistical analysis. A 'dashboard' program enables the user to remotely monitor the mice in the PhenoTyper cages and the progress of all trials, and to perform remote diagnostics of the system. After the 6 days in the PhenoTyper, mice are transferred to new home cages containing a running wheel. For the first 5 days, mice are left to habituate to these cages under a standard light-dark regime. During the final 5 days lights remain off and the free running rhythm of mice is assessed.

Ultimately, in an automated process, the behavioral measures of each individual mouse are aggregated into a phenogram, to allow comparison with a reference mouse (i.e. a wild type strain).

The behavioral measures currently being obtained in the high throughput screening protocol will be compared to known behavioral abnormalities of these strains, as measured with several established stand-alone tests. During the conference presentation I will address whether the current automated high throughput screening protocol allows reliable discrimination of common inbred lines and whether some behavioral measures can be validated by the standard stand alone testing protocols.

## References

1. Crabbe, J.C., Wahlsten, D., Dudek, B.C., 1999. Genetics of mouse behavior: interactions with laboratory environment. Science (New York, N.Y 284, 1670-1672.
2. de Visser, L., van den Bos, R., Kuurman, W.W., Kas, M.J., Spruijt, B.M., 2006. Novel approach to the behavioural characterization of inbred mice: automated home cage observations. Genes Brain Behav 5, 458-466.

[1]*The Neuro-Bsik Mouse Phenomics consortium consists of the following academic and commercial partners: Erasmus University Medical Center, represented by the departments of Neuroscience (Prof. Dr. C.I. de Zeeuw, Dr. Y. Elgersma, Prof. Dr. J.G.G. Borst) and Cell Biology and Genetics (Dr. ir. N.J. Galjart, Prof. Dr. G.T.J. van der Horst). Vrije Universiteit Amsterdam, represented by the departments of Functional Genomics (Prof. Dr. M. Verhage), Experimental Neurophysiology (Prof. Dr. A.B. Brussaard) and Molecular and Cellular Neurobiology (Prof. Dr. A.B. Smit). Utrecht University, represented by the department of Animals, Science and Society (Prof. Dr. B.M. Spruijt). University of Amsterdam, represented by the Swammerdam Institute for Life Sciences (Prof. Dr. C.M.A. Pennartz). Netherlands Institute for Neuroscience (Dr. C.N. Levelt). Synaptologics BV. Noldus Information Technology.*