Home-cage Automated Cognitive Phenotyping in Mice

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Mouse mutant lines constitute promising translational models to investigate mental illness and to develop new therapeutic approaches. As advances in mouse genetics technology allow to engineer mouse models carrying complex genetic variants, there are now several large-scale projects, world-wide, to create collections of mouse mutants based on relevant phenotypic data [1-5].

However, the vast repertoire of mouse models is not currently paralleled by a correspondingly rich set of phenotyping methods. A "phenotype" can be defined as any functional or anatomical expression of the organism's genotype and its interactions with the environment. Phenotypic assessment is required to build any link between genes and mental functions, but classical research on mental health using rodent models has mainly relied on coarse assessments (such as rudimentary behavioral analyses), which have limited translational value.

Moreover, while most biological sciences are progressing towards a holistic approach to investigate the complexity of organisms (i.e., "systems biology" approach), mouse functional genomics has embraced a more reductionist strategy that focuses on intermediate phenotypes (endophenotypes) [6]. The investigation of single endophenotypes has been a powerful approach in mouse genetics but, in many cases, it was not followed by a better understanding of human disease susceptibility [7, 8]. The reason for this poor translational result is that the genetic analysis of mouse phenotyping does not necessarily rely on an increase of isolated behavioral analyses. Instead, the success of large-scale genetic investigations by using laboratory animals very much depends on the ability: (i) to integrate meaningful and multi-dimensional comparable traits; (ii) to monitor time-variable phenotypes; (iii) to develop novel technologies that detect and analyze phenotypes in home-cage environment. In addition, phenotypic data must become easily accessible for the whole scientific community [9] to promote the continuous integration with new scientific information.

Thus there is an urgent need for new investments in basic and integrated robust phenotyping technologies [10].

Here I will present a series of behavioural/cognitive measures in mice that were derived with automated behavioural protocols. Mice were subjected to a long-term investigation in their home-cage environment. A novel operant wall was installed in regular mouse cages. The wall is equipped with two lateral hoppers which are connected to pellet dispensers and one central hopper that is used by the mouse to self-initiate the trial. All animals were trained on a timing task that required them to judge when to switch from one feeder to another for reward maximization [11]. In a given trial, reward could occur at one of the two feeding locations with some predetermined probability and after different corresponding latencies. The optimal performance in this task requires animals to have an accurate representation of time intervals (i.e., short and long intervals), the endogenous uncertainty about these intervals, and the exogenous uncertainty (i.e., probability of reward occurring at two different locations). We have also introduced long and short probe trials with different probabilities.

After a standard training, all mice were subjected to three experimental phases starting with ratio 1:2 (3 s vs. 6 s). In each phase we introduced probe (not rewarded) trials, with different probabilities, to test the endogenous uncertainty according to risk exogenous assessment. During the first experimental phase (Exp. 1) short probe (S_p) and long probe (L_p) trials were introduced with the same conditional probabilities, $P(S_p|S) = P(L_p|L) = 0.2$. In the second experimental phase (Exp. 2) $P(S_p|S) = 0.5$ and $P(L_p|L) = 0.2$, and vice versa for the third one. Furthermore, the analysis of the probe trials along the experimental phases allows to understand and quantify the mice perception of the changes in the exogenous assessment.

The analysis of timing behaviors across different experiments and across 24 hours has shown significant circadian effects on some behavioral measures. Moreover, we present evidence that daily variations in

behavioral performance correlate with circadian-sleep processes. In particular, when the pressure of sleep is thought to increase, the behavioral performance decreases.

Comprehensive phenotyping of thousands of mutant mouse strains (i.e., across mouse genetics consortia around the world) will provide an unprecedented volume of data in the future. Current bioinformatic tools, such as *ontologies* [12-15], are designed to provide a repository for multimodal data but are not well-suited for integrated analyses between genome and detailed and dynamic phenome information. In this project we will investigate, by means of our multi-dimensional experiments, novel computational strategies to investigate the link between genotypes and phenotypes by considering new factors, i.e., time-changing variables.

Through the symposium we will discuss the advantages and the current challenges of realizing fully automated systems for behavioral screens in mice.

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