SUMMARY

Behavioral phenotyping of complex traits in inbred and mutant mice

Neurological pathologies and psychiatric disorders are commonly detected through distinct human behavioral abnormalities. From a medical perspective, the understanding of the underlying biological mechanisms involved in those behaviors is essential. The complexity of both these abnormal behaviors and the biological mechanisms requires the use of animal models and appropriate phenotyping systems.

Common behavioral tests in mice are based on locomotor activity measures and are designed to segregate behaviors over a short period of time (5 to 60 min). Thus, to describe the effect of a particular genetic background, a genetic mutation, or a drug on behavior, a battery of tests is required to tap into different aspects of behavior such as motor, sensory, cognitive and circadian functions. Those sets of experimental procedures are time consuming. Moreover, repeated human-animal interactions, environmental effects and other technical differences between laboratories are sources of variation in the results that lead to non-replication and difficulty in the interpretation of results. Consequently, there is growing consensus that behavioral testing needs a boost toward automation to increase its effectiveness and standardization in the study of animal models of human diseases and therapeutic developments.

In the last decade, new behavioral technologies have emerged, allowing automated observation of animals in their home cage over long periods of time (e.g., several consecutive days). Automation of observation allows repetitive, objective, and consistent measurement with minimal human-animal interactions. Furthermore, continuous recording allows investigation of multi-dimensional aspects of behavior like habituation, and baseline and challenged behavior, offering the possibility to study longitudinally the progression or change of behavior. This is of utmost relevance to the study of models of neurological, psychiatric, and neurodegenerative disorders.

In **Chapter 2,** we characterized complex behavioral responses indicative of avoidance learning in mice, using an unsupervised automated high throughput system. We adopted an assay exploiting the natural tendency of mice to develop a preference for one of two shelter entrances, by automatically detecting shelter entrance preference. Next, when the preferred entrance was used to enter the shelter, the animal was sanctioned by a mild aversive stimulus (illumination of the shelter with bright light). This learning paradigm specifically addressed cognitive aspects of avoidance behavior and produced a wealth of information on other aspects of behavior. Using this assay, we screened 8 inbred strains and a panel of 43 different mutants, and identified a new candidate gene, *specc1/cytospinB*, involved in avoidance learning. Our data show that the complex adaptive behavioral response of mice can efficiently and successfully be detected, analyzed and visualized even in large cohorts of (mutant) mice. Various inbred strains and single gene mutants exhibited marked quantitative differences in distinct aspects of this behavioral task.

Chapter 3 investigated the potential of *Munc18-1* haploinsufficiency in mice as a model for early infantile epileptic encephalopathy (EIEE), also known as Othahara syndrome. *De novo* heterozygous mutations in the human *MUNC18-1* gene, *STXBP1* are suspected to cause severe intellectual deficits with or without epileptic seizures. *MUNC18-1* protein is essential for regulatory functions of synaptic vesicle secretion in mammalian synapses, a homozygous deletion of

Munc18-1 results in a postnatal death. Using an extensive test battery, including high-throughput home cage behavioral phenotyping and a wide range of classical behavioral tests, we investigated the behavioral effects of *Munc18-1* haploinsufficiency in mice. Heterozygous (HZ) *Munc18-1* mice showed no obvious epileptic seizures or cognitive impairment. However, despite the high anxiety level observed, HZ mice exhibited a lower fear responses and faster extinction. *Munc18-1* HZ mice were, however, more anxious and showed a more proactive coping strategy compared to their WT litter mates when facing a more severe stressor. The reduced amount of *MUNC18-1* protein appears to be sufficient to maintain most of the cognitive function in the mice.

In **Chapter 4**, we focused on the characterization of a potential mouse model for Major Depressive Disorder (MDD). Genome-wide association studies (GWAS) have suggested a role for a non-synonymous exonic variation in the presynaptic gene *PCLO* in MDD. We investigated the *PCLO* variation (S4814A) and its effects on a molecular, cellular and behavioral level in the highly homogeneous background of C57BL/6J inbred mice. Knock-in mouse model expressing the $Pclo^{SA/SA}$ variant showed an increased synaptic Piccolo level and a 30% increased excitatory synaptic transmission in cultured neurons. However, anxiety, cognition and depressive-like behavior were normal in $Pclo^{SA/SA}$ mice. The fact that MDD is a multifactorial disease, brought on by a combination of many genetic and environmental factors might be a reason why $Pclo^{SA/SA}$ mice did not show a strong behavioral phenotype. However, the molecular changes we observed might slightly increase the risk for MDD under certain circumstances.

Historically, most of the studied genes were discovered after clinical observations in dramatic phenotype alterations, leaving the majority of genes in the genome unstudied. Thus in **Chapter 5**, we generated 5 randomly mutated mouse strains (*Dpp10*, *Fgf13*, *Kcnd2*, *Ttc39c* and *Ubn1*), using germlines carrying the Sleeping Beauty transposase together with a transposon. Those 5 strains were behaviorally phenotyped using a novel screening approach involving avoidance learning. The home cage-based system used to screen spontaneous and avoidance learning behavior described in e.g. Chapter 2, revealed the high sensitivity of spontaneous behavior to gene modifications. Moreover, we showed the involvement of *Ubn1*, a subunit of the HUCA histone chaperone complex, in the formation of simple associative memory. Studying 5 random functionally unknown genes and finding out that one of them is involved in cognitive processes, might suggest that a large proportion of genes in the genome are directly or indirectly related to cognition, which support the high genetic complexity of brain function.

Finally, **Chapter 6** summarizes the major results of 4 years of work on behavioral phenotyping and discusses the added value of high-content and high-throughput behavioral phenotyping to investigate mouse models of neurologic pathologies and genes involved in cognitive functions. Overall, the recently developed automated home cage phenotyping systems are expected to substantially improve and complement the study of ethologically valid behavior in mice. While additional validation is still required, the studies reported in this thesis do show that automated phenotyping is turning into an essential tool in the characterization of genetically modified mice for higher translational value of disease/disorder models.