

SMP: Mammalian Models**Project: German Mouse Clinic - Neurological Screen**

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Introduction

Neurological dysfunction results in a wide variety of disorders ranging from impaired movement to severe mental illness. Studying the neurobehavioural phenotype of mutant mice is a powerful tool to understand the neural basis of behaviour and the pathophysiology of neurological and psychiatric disorders. Hundreds of different genes expressed in the central nervous system have been targeted in transgenic and knockout mice. The phenotyping of these mice is becoming a serious bottleneck in the development of animal models; an exponentially increasing number of genotypes is created, but the behavioural and neurological phenotyping is often rudimentary or abandoned completely.

Comparison of the mouse and human brain transcriptomes shows a good correlation for highly expressed genes in both transcript identity and abundance. Therefore, screening of mice potentially boosts the understanding of human neurological disorders. The main aim of the neurological screen of the GMC is to provide well-characterized mouse models for neurological diseases, to investigate the *in vivo* consequences of the mutations, and to allow for therapeutic trials (Gailus-Durner et al, 2005).

Project Status

The neurological screen offers a comprehensive and standardized neurological phenotyping of mutant mice for the assessment of neurological and neuromuscular functions. It is performed in three steps.

Primary screen. Mice are analyzed according to a modified **SHIRPA** (Smithkline Beecham, MRC Harwell, Imperial College, the Royal London hospital phenotype assessment) protocol where a battery of behavioral tests is carried out (Hatcher et al, 2001). This primary observation screen is proposed as a rapid and semi-quantitative screening method for analysis of abnormal phenotypes in mice. The test parameters contribute to an overall assessment of muscle, motor neuron, spino-cerebellar, sensory and autonomic functions. In addition, quantitative measurement of the forelimb **grip strength** is performed as a primary assessment of muscle function. We also examine **lactate** levels in the blood of mice to draw conclusions about energy metabolism.

In order to further develop and standardize the tests, several inbred strains were tested as well. The marked relevance of the genetic background for many neurological parameters could be demonstrated.

Secondary screen. The secondary screen is performed in selected mice based on the results of the primary screen after consultation with the mouse provider. It comprises the assessment of motor coordination on the RotaRod, the analysis of skilled reaching by the staircase test and the rating of gait abnormalities applying the footprint analysis. The performance of mutant mice on the **Rotarod** provides information about motor coordination and balance as well as basic motor learning skills dependant upon the protocol used (Fig.1).

Skilled reaching movements with the forelimbs are necessary in the **staircase test**, where the mice have to grasp for food pellets from a narrow staircase (Fig.2). This test allows for the detection of extrapyramidal motor dysfunction as well as unilateral neurological deficits, e. g. brain lesions in stroke models. The newly established **footprint analysis** allows the detection of abnormal gait characteristics, e. g. in cerebellar disorders.



Fig 1: Rotarod apparatus for the assessment of motor coordination and balance.

Tertiary screen. The tertiary screen comprises two invasive, technologically advanced and time-consuming methods. **Telemetric electroencephalography** is performed to record electrical activity of the cerebral cortex. The employment of a telemetric device allows the investigation of awake mice not compromised by outgoing wires, which is a big advantage as compared to conventional systems. We already applied this method successfully to characterize a mouse model for epilepsy. This model is now intended for a project on the evaluation of antiepileptic drugs.

Muscle biopsies are performed in selected mouse strains if there is evidence for a myopathy or a mitochondrial disease. Quadriceps muscle is dissected from sacrificed mice, and muscle morphology is done using an array of histological and histochemical stains. A particular high value is set on the detection of mitochondrial abnormalities.



Fig 2: Staircase experiment to test skilled reaching.

Results

We have now completed the validation of our methods in several inbred mouse strains that are usually used for the generation of mutant lines. We found large differences in the neurological phenotype of these mice enabling the

interpretation of results in our mutant lines (Gailus-Durner et al, 2005; Schneider et al, in preparation).

Until now, 35 mutant mouse lines were phenotyped in the primary neurological screen. In some of the mutant lines known phenotypes were confirmed, but in one third of the lines additional phenotypes could be found. Some of the results are summarized below.

MML1 gene-trap mutant of a transcription factor involved in steroid signalling (Miz1)

In addition to some expected behavioral variations in these mice also neurological alterations were found. Male mutant mice displayed altered gait characteristics and were impaired in the wire manouvre test. These findings pointed towards a sex-specific defect in motor coordination and muscular function and confirmed an influence of steroid signalling on neuromuscular functions in these mice.

MML2 ENU-generated MML displaying tremor (ABE17)

Mutant mice exhibited an abnormal gait and different tail elevation. Forelimb grip force was reduced hinting towards a muscular dysfunction. In the rotarod experiment we observed a reduced latency of mutant mice on the rod. Further analyses for the discrimination of the pathways involved are in progress.

MML3 knockout mouse model for an epileptic seizure disorder (SePP)

These mutant mice showed action-tremor and gait abnormalities. In addition to these cerebellar features also a strong decrease in locomotor activity was detected. This hints towards alterations in the dopaminergic system as well. Several neurological reflexes were impaired, too. In righting reflex, contact righting reflex, negative geotaxis and wire manoeuvre the mutant mice performed worse than controls.

MML4 knockout mice with an immunological disorder (Elastin)

In these mice several neurological parameters were altered. Male knockout mice showed significant changes in the visual placing test which could be due to an impaired visual ability. In contrast to female controls, female knockout mice displayed a lower locomotor activity and a slower movement (Spontaneous Behavior). Therefore further analyses are projected in order to clarify the neurological phenotype detected.

MML5 knockout of a gene involved in fatty acid metabolism (MFP2)

Both male and female mutant mice had a changed somatosensory discrimination (visual placing test) and difficulties in their body coordination. An impaired motor performance was detected in the negative geotaxis test. Generally, balance and grip strength are required to keep the mouse's body suspended on the grid. Further investigation of rotarod performance as well as grip strength measurement revealed significant impairment of the mutant mice. Taken together these results point towards a defect in the cerebellum or in the spinocerebellar pathways.

MML6 knockout of a cytoskeleton protein implied in neuronal lineage specification (vimentin)

Cerebellar defects were already described for this MML manifesting itself in impaired rotarod performance. We could confirm in our analyses an increase in locomotor activity in these mice. In addition to the above mentioned we also detected a significant decrease in forelimb grip force. A possible neuromuscular defect in these mice has to be examined in our secondary screen.

A special focus of our research is the analysis of mitochondrial disorders. Mitochondria provide cellular energy and defects of energy metabolism are involved in a variety of human diseases. These disorders manifest preferentially in

tissues with high aerobic demand such as brain and muscle. Several mitochondrial mouse models are currently being investigated in the neurological screen. The most advanced one is the gene trap-generated knock-out mouse for Tim23, an essential protein for the mitochondrial import machinery. While homozygote mice are not viable, heterozygote mice show an aging phenotype with reduced life span, kyphosis and skin changes. Western blot shows a 50% reduction of the Tim23 protein. Other mitochondrial mice are under investigation.

Outlook

The array of neurobehavioural phenotyping methods is continually expanded and improved. The methodological aim is a comprehensive compilation of tests for the standardized analysis of neurological defects. New methods will again be validated in the wild-type strains used. Moreover, we work on a "short protocol" for a rapid neurological system survey.

These methodological innovations will directly benefit the neurological screening of mice from our collaboration partners inside and outside the NGFN. Among others, we have interesting models for a myopathy (muscle biopsies already performed) and for an epileptic seizure disorder (electroencephalography already performed) under investigation.

The variety of mitochondrial mouse models that is under investigation will hopefully contribute to the understanding of mitochondrial pathophysiology and lead to models for therapeutic trials.

Lit.: 1. Gailus-Durner V et al. Introducing the German Mouse Clinic: open access platform for standardized phenotyping. Nat Methods. 2005 Jun;2(6):403-404. 2. Hatcher JP et al. Development of SHIRPA to characterise the phenotype of gene-targeted mice. Behav Brain Res. 2001 Nov 1;125(1-2):43-47. 3. Schneider I et al. Systematic, standardized and comprehensive neurological phenotyping of inbred mice strains in the German Mouse Clinic. In preparation.