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Systematic, standardized and comprehensive neurological phenotyping of inbred mice strains in the German Mouse Clinic

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Abstract

Neurological and psychiatric disorders are among the most common and most serious health problems in developed countries. Transgenic mouse models mimicking human neurological diseases have provided new insights into development and function of the nervous system. One of the prominent goals of the German National Genome Research Network is the understanding of the in vivo function of single genes and the pathophysiological and clinical consequences of respective mutations. The German Mouse Clinic (GMC) offers a high-throughput primary screen of genetically modified mouse models as well as an in-depth analysis in secondary and tertiary screens covering various fields of mouse physiology. Here we describe the phenotyping methods of the Neurological Screen in the GMC, exemplified in the four inbred mouse lines C57BL/6J, C3HeB/FeJ, BALB/cByJ, and 129S2/SvPas. For our primary screen, we generated "standard operating procedures" that were validated between different laboratories. The phenotyping of inbred strains already showed significant differences in various parameters, thus being a prerequisite for the examination of mutant mouse lines.

Keywords: Neurological phenotyping; Inbred mouse strains; SHIRPA; Grip strength; Rotarod; EEG

1. Introduction

Studying the neurobehavioural phenotype of transgenic mice and their inbred background strains 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 (Hafezparast et al., 2002; Watase and Zoghbi, 2003). Comparison of the mouse and human brain transcriptomes shows a good correlation for highly expressed genes in both transcript identity and abundance (Fougerousse et al., 2000). Therefore, screening of mice with respect to neurological disorders potentially offers an understanding of aetiology and pathogenesis of the human nervous system. For the comparison of neurological phenotyping data, standardized protocols were developed only recently, including a neurodevelopmental screening (Dierssen et al., 2002) and a behavioural phenotyping of mice in pharmacological and toxicological research (Karl et al., 2003). The Mouse Phenome Project (http://aretha.jax.org/pub-cgi/phenome/mpdcgi?rtn=docs/home) promotes the quantitative phenotypic characterization of a defined set of inbred mouse

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strains under standardized conditions (Paigen and Eppig, 2000; Bogue, 2003; Bogue and Grubb, 2004; Grubb et al., 2004). Several inbred strain comparisons have been published (Balogh et al., 1999; Tarantino et al., 2000; Volkar et al., 2002). Transgenic and knockout databases with behavioural profiles of mouse mutants are described by Anagnostopoulos et al. (2001).

The German Mouse Clinic (GMC, www.mouseclinic.de) has the goal to develop and optimize a battery of different test parameters in different disciplines. The GMC was established as a part of the German NGFN (National Genome Research Network) and in collaboration with the pan-European Eumorphia consortium (www.eumorphia.org) and offers a systematic, standardized and comprehensive phenotyping of mutant mice to identify and characterize mouse models for human diseases. The GMC comprises thirteen screens covering neurology, behaviour, nociception, dysmorphology, immunology, clinical chemistry, allergy, steroid metabolism, energy metabolism, lung function, expression profiling, cardio-vascular function and pathology (Gailus-Durner et al., 2005). A primary screening of a mutant mouse line (MML) includes all screens; each mouse is tested consecutively in several laboratories.

The neurological screen of the GMC established a standardized primary screening including validation of the data. This comprises the comparison of results of testing on selected inbred mouse strains and/or selected mutants at more than two Eumorphia laboratories (Green et al., 2005). In the primary neurological screening a modified SHIRPA protocol (Rafael et al., 2000; Rogers et al., 1997, 2001) and a grip strength analysis is used that allows for a high-troughput screening of MMLs. Dependant upon results of this primary screen and due to specific questions, additional tests can be carried out for further assessment of neurological functions in a hierarchical way (van der Staay and Steckler, 2001). The secondary screen comprises a rotarod analysis, and the tertiary screen a staircase test and, if applicable, electroencephalography (EEG). We will further broaden this arsenal of methods in the future. Behavioural tests such as the modified hole board test, the open field test and the Morris–Water–Maze test are performed in the complementing Behavioural Screen of the GMC (www.mouseclinic.de). The main aim of the neurological screen is to provide well-characterized mouse models for known neurological diseases with known gene defects (generated by transgenic, knockout and gene trap approaches), to investigate the in vivo consequences of the mutations, and to allow for therapeutic trials. In this paper we present our large spectrum of methods using inbred mouse strains C57BL/6J (C57), C3HeB/FeJ (C3H), BALB/cByJ (BALB), and 129S2/SvPas (129/SvP) as test models**.** Only the most significant results are presented. The choice of the given inbred strains was based on the frequent use of these mice in transgenic research (Gerlai, 1996; Taketo et al., 1991). In conclusion, we want to highlight the importance of well-established phenotyping protocols and the investigation of baseline data from inbred strains to the diligent phenotype analysis of MMLs. These baseline data facilitate the evaluation of differences found in numerous MMLs analyzed of different or mixed background.

2. Material and methods

2.1. Animals

Four inbred strains of male mice were used: C57BL/6J (C57) from Charles River, Germany, C3HeB/FeJ (C3H) from GSF Munich, Germany, BALB/cByJ (BALB) from Jackson Lab, USA, and 129S2/SvPasIco (129/SvP) from Charles River, France. All tests of the primary (SHIRPA, grip strength) and secondary (rotarod) neurological screen were performed in 10-week-old mice. The mice were caged in an animal colony maintained on a 12:12 h regular light–dark cycle. All experiments were done according to the German laws on animal protection and with permission from the "Regierung von Oberbayern".

2.2. Primary screening: SHIRPA protocol

The SHIRPA (Smithkline Beecham, MRC Harwell, Imperial College, the Royal London hospital phenotype assessment) protocol (Irwin, 1968) is a rapid, comprehensive, and semi-quantitative screening method for qualitative analysis of abnormal phenotypes in mice. In the neurological screen within the GMC, it is used in a modified form (Rafael et al., 2000; Rogers et al., 1997, 2001). We carried out 23 test parameters that contribute to an overall assessment of muscle, lower motor neuron, spinocerebellar, sensory and autonomic function (Online supplement Table S I). A "standard operating procedure" (SOP) for the SHIRPA test was generated, tested in the four inbred mouse lines and validated between different laboratories.

2.2.1. Behaviour in the viewing jar

After weighing of the mouse, neurological assessment starts with the observation of the undisturbed animal's behaviour in a glass-viewing jar (Ø 11 cm) for 3 min. Items of interest are body position, tremor, palpebral closure, coat appearance, whiskers, and excessive lacrimation or defecation.

2.2.2. Behaviour in the arena

After transfer into an arena (36 cm × 20 cm), each mouse is tested for transfer arousal, locomotor activity and gait along with any bizarre or stereotyped motor behaviour. For locomotor activity a grid (3 × 5 squares) on the floor of the arena is used. During 30 s the number of squares are counted that the mouse enters with all four paws. Additionally, tail elevation, touch escape, positional passivity, and skin colour are scored.

2.2.3. Behaviour in or above the arena

Then, each mouse is lifted vertically up at mid-tail for 15 cm and curling of the trunk as well as possible grasping of the hind paws is observed. Pinna and corneal reflexes are tested by approaching the pinna and the eye, respectively, with the tip of a clean cotton swab. For the air-righting reflex, the animals are held horizontally in an inverted position 30 cm above a 10-cm foam bed, and are then released. If the mice show any obvious movement abnormalities before and at the transfer to the arena,