



**EUMORPHIA FINAL REPORT
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Summary

With the completion of the mouse genome sequence, one of the key goals for functional genomics is the creation of a series of mutant alleles for every mammalian gene. Large-scale mutagenesis efforts are underway using both gene-driven and phenotype-driven approaches to generate this resource of mouse mutants. An even greater challenge will be the determination of the phenotypic outcomes of each mutation. A vital element of this endeavour will be to develop standardised phenotyping platforms that allow for reproducibility of test outcome over both time and place. The Eumorphia programme is a consortium of 18 research institutes from across Europe working on establishing new methods in phenotyping with a focus on improving and standardising phenotyping platforms for the mouse. Eumorphia has developed a new robust primary screening platform, EMPReSS - European Mouse Phenotyping Resource for Standardised Screens. This primary screen incorporates over 150 new SOPs and associated annexes and appendices, many validated on a cohort of inbred strains across a number of laboratories. EMPReSS covers all of the major body systems, as well as generic approaches in imaging, pathology and gene expression. We have developed a database and web-based resource for the visualisation, searching and downloading of the EMPReSS SOPs and other documents that constitute EMPReSS (www.empress.har.mrc.ac.uk). We have also developed a phenome database to hold baseline data from the validation of the SOPs on inbred strains (www.euromorphia.eu). The availability of new standardised screens and associated informatics structures and tools will be a vital underpinning for a systematic and rational functional annotation of the mouse genome.

Introduction

The Eumorphia consortium has developed a comprehensive, robust and validated phenotyping platform for the mouse. Initially, we have focused on the development of a phenotyping platform encompassing first-line screens, many of which are accessible to a typical mouse genetics lab. The new screen, EMPReSS (European Mouse Phenotyping Resource for Standardised Screens) encompasses over 150 SOPs and associated annexes and appendices, many of which have been validated within and across laboratories of the Eumorphia consortium (www.empress.har.mrc.ac.uk). Such a screen will be a valuable start-point for systematic phenotyping of the growing mouse mutant collections, as we move from mutant generation in the mouse to functional annotation.

With the completion of the genome sequence of several mammalian organisms, considerable attention is now focused on the functional annotation of these genomes and establishing the relationship between gene and phenotype. The most versatile organism to study mammalian gene function is the mouse as there is available an extensive toolkit for modifying the genome and specific genes. There are two distinct approaches to determining gene function in the mouse – gene-driven and phenotype-driven¹.

In the gene-driven approach, a defined lesion in the mouse genome is the start-point for an analysis of the resulting phenotype. Gene-driven approaches include gene-traps, targeted traps and both targeted knock-out and knock-in mutations^{2,3,4,5}. Targeting constructs can be manipulated so that mutations are conditional and the phenotype of the mutation can be explored in a time-dependent or tissue-specific manner. Moreover, it is entirely feasible for gene-driven mutagenesis approaches to be scaled in order to introduce mutations in every gene in the mouse genome. There has been much recent discussion on the generation of mutation resources, both null and conditional, for all mouse genes^{6,7}. Two such programmes, EUCOMM (the European Conditional Mouse Mutagenesis Programme) and NorCOMM (the North American Conditional Mouse Mutagenesis Programme), focusing on generating libraries of ES cells carrying conditional mutations, are now funded and underway. It can be expected that additional programmes funded through the KOMP (the Knockout Mouse

¹ Brown SDM, Balling R (2001) Systematic approaches to mouse mutagenesis. *Curr. Opin. Genet. Devel.* 11: 268-273

² Stanford WL, Cohn JB, Cordes SP (2001) Gene-trap mutagenesis: past, present and beyond. *Nat. Rev. Genet.* 2: 756-768

³ Hansen J, Floss T, Van Sloun P, Füchtbauer E-M, Vauti F et al. (2003) A large-scale, gene-driven mutagenesis approach for the functional analysis of the mouse genome. *PNAS* 100: 9918-9922

⁴ Branda CS, Dymecki SM (2004) Talking about a revolution: the impact of site-specific recombinases on genetic analyses in mice. *Dev. Cell.* 6: 7-28

⁵ Carlson CM, Largaespada DA (2005) Insertional mutagenesis in mice: new perspectives and tools. *Nat. Rev. Gen.* 6: 568-580

⁶ Auwerx J, Avner P, Baldock R, Ballabio A, Balling R et al. (2004) The European dimension for the mouse genome mutagenesis programme. *Nature Genetics* 36: 925-927

⁷ Austin CP, Battey JF, Bradley A, Bucan M, Capecchi M et al. (2004) The Knockout Mouse Project. *Nature Genetics* 36: 921-924

Project) initiative in the US will follow. In addition to gene-driven approaches that target individual genes, it is also possible to engineer larger chromosomal changes to investigate the function of gene clusters⁸.

The phenotype-driven approach aims to search large collections of randomly mutagenised mouse genomes, usually produced by ENU mutagenesis, for phenotypes of interest irrespective of the underlying lesion that may have been responsible^{9,10,11}. Thus the phenotype of interest is the start-point of the study, and having discovered an interesting mutant phenotype the underlying gene is identified and investigated further. Phenotype-driven approaches do not make any a priori assumptions about the relationship between gene and phenotype, in contrast to the gene-driven approach where the discovery of relevant phenotypes can pose difficulties. In addition, ENU introduces point mutations and has the capacity to reveal many of the gene-phenotype relationships at an individual locus by the introduction of a range of null, hypomorphic, gain-of-function and dominant negative mutations. Moreover, recently, the use of ENU for gene-driven approaches has been demonstrated by the creation of parallel archives of frozen sperm and DNA from ENU mutagenised males^{9,10}.

Taken together, through gene-driven and phenotype driven approaches, it is clear that the mouse offers a rich basket of tools and approaches to generate mutations. Underpinning this diversity is the recognition of the utility of a series of mutant alleles at every gene in the mouse genome. Such a resource would enable us to explore fully gene-phenotype space and to provide a wider collection of disease models for translating gene function in the mouse to the study of disease genetics in the human. However, such a programme is dependent not only on the creation of the relevant mutants but also on the tools to investigate phenotype. Irrespective of whether a gene-driven or phenotype-driven approach is adopted, the ability to phenotype the mouse is pivotal to a successful outcome.

Comprehensive phenotyping platforms for any species depend upon a number of features. Firstly, they require the development of approaches to study, document and measure aspects of all body systems. Secondly, they require standardisation to ensure that the phenotyping protocols are reliable both within and between laboratories and over time. Such standardisation requires validation by testing and assessment between mouse genetic

⁸ Coghill E, Hugill A, Parkinson N, Davison C, Glenister P, et al. (2002) A gene-driven approach to the identification of ENU mutants in the mouse. *Nature Genetics* 30: 255-256

⁹ Nolan PM, Peters J, Strivens M, Rogers D, Hagan J et al. (2000) A systematic, genome-wide, phenotype-driven mutagenesis programme for gene function studies in the mouse. *Nature Genetics* 25: 440-443

¹⁰ Hrabé de Angelis M, Flaswinkel H, Fuchs H, Rathkolb B, Soewarto D et al. (2000) Genome-wide, large-scale production of mutant mice by ENU mutagenesis. *Nature Genetics* 25: 444-447

¹¹ O'Brien TP, Frankel WN (2003) Moving forward with chemical mutagenesis in the mouse. *J Physiol* 554: 13-21

centres. Finally, as part of the validation process it is important to document where possible the factors that impinge upon the robustness of phenotype outcome using any phenotyping protocol, which may range for example from animal house environment to equipment design.

There already has been some effort to develop novel, high-throughput phenotyping platforms for studying mouse mutants. One example is the SHIRPA screening protocol¹², which focused on assays in a variety of functional domains including neurological, behavioural and sensory phenotypes amongst others. However, these platforms have by and large been restricted in their phenotype coverage and importantly have not been developed using a process of systematic validation to ensure they are robust across time and place.

Standardisation of phenotyping is key to the challenge of developing a comprehensive functional annotation of the mouse genome. To take but one simple example, if investigators are not able to reliably compare phenotype outcome for two different alleles in the same gene, then any interpretation of similarities or differences that might impact upon our understanding of gene function will be fraught with difficulties. It will thus be important that standardised methods are adopted by centres undertaking large scale efforts to phenotype mutant mice collections. Equally, it is vital that such standardised procedures are accessible to and operable by smaller laboratories. The aim is to generate a set of standardised procedures that will become a comprehensive reference point for mouse phenotyping.

Methods

Working groups to develop and standardise phenotyping methods

The development and validation of new methods for mouse phenotyping was distributed across a number of working groups, each group comprising a number of mouse genetics centres from around Europe and covering a particular body system (Table 1). In addition, we established working groups in several generic areas that are important to mouse phenotyping. One working group examined the impact of animal house conditions on phenotype assessment and considered what standardisation exists and could be introduced across the various centres. Additional working groups developed new protocols in imaging, pathology and gene expression. Finally, an Informatics working group was established to underpin the various data structures that would be needed to foster dissemination of the resulting methods. Each working group also invited additional expertise from clinicians and physiologists where appropriate to assist with the development of phenotyping methods. The initial emphasis for each working group was to develop and standardise phenotyping

¹² Rogers DC, Fisher EMC, Brown SDM, Peters J, Hunter AJ, Martin JE (1997) SHIRPA - A proposed protocol for the comprehensive behavioural and functional analysis of mouse phenotype. *Mammalian Genome* 8: 711-713

methods that would act as first-line screens for the mouse. Many centres were represented on several working groups fostering cross-talk between the work packages and ensuring that the developed methods were suitably integrated.

The flow of work for the development of SOPs is diagrammed in Figure 1. Briefly, each working group met to determine those tests that were suited to first-line screening, and develop draft SOPs for the selected tests (Status level 1). We distinguished between Procedural and Data Generating SOPs. Procedural SOPs include SOPs that are components of Data Generating SOPs. For example, blood collection is provided as a distinct Procedural SOP but is used extensively within many Data Generating SOPs. Draft SOPs were tested on a selected group of inbred strains (see below) within the constituent laboratories of the working group. Only Data Generating SOPs were the focus of validation. Validation might occur within a single laboratory to ensure operational consistency of the developed SOP. However, we endeavoured with many SOPs to validate between laboratories and this process continues. Results were compared and where appropriate SOPs modified or further developed to ensure consistency. If necessary, additional rounds of validation took place. Following validation, each SOP was reviewed by the Eumorphia Project Office team for accuracy and consistency with the established SOP format (Status level 2; see also below). Finally, the SOP was reviewed and signed-off by a Eumorphia scientist outside of the working group (Status level 3) before upload to the EMPReSS website.

Validation using inbred strains and mutants

Four inbred strains - C57BL/6J, C3HeB/FeJ, BALB/cByJ, 129S2/SvPas - were chosen to assist with validation of SOPs. The selection is inevitably somewhat arbitrary but all four strains can be found in the key group of strains chosen by the Mouse Phenome project (www.phenome.jax.org) for phenotype characterisation. We therefore expect the phenome data generated through the EMPReSS screen validation efforts to be a useful adjunct to mouse phenome databases. C57BL/6J is a widely used inbred strain that is often employed for the construction of congenic lines and the comparison of mutant phenotypes. We chose a 129 line, 129S2/SvPas, as part of the group, reflecting the wide use of 129 inbred mice in mutant generation, but recognise that there are major differences between the various 129 sub-lines. BALB/c along with C3H is frequently used in ENU mutagenesis programmes. Many SOPs were validated across all four strains, while some SOPs employed a sub-set of this group. For some SOPs additional validation took place by testing on relevant mutant lines.

Primary and primary extended screens

First-line or primary screens represent screens which can be carried out at relatively high-throughput and represent those tests that can be applied in a comprehensive manner to a significant number of mouse mutants. However, in developing new methods for primary screens we recognised the utility of dividing the SOPs into two classes:

- **Primary** protocols are simple to apply and require little specialist equipment.
- **Primary extended** protocols give further information on the phenotype but require more specialised skills or equipment.

Most laboratories would be expected to be able to undertake Primary screens, but might require assistance or access to equipment to undertake Primary Extended screens.

Design and implementation of SOP

A comprehensive SOP format (Figure 2) was devised that incorporates all technical instructions necessary to perform the phenotyping test. Importantly, the format also allows the systematic integration of the assay with the necessary informatics solutions to link the SOPs to defined phenotype outcomes and we discuss below the integration of the SOP with phenotype ontologies. Some SOPs are accompanied by appendices, which provide more detailed descriptions of part of the procedure or the analysis of the procedure. There are also annexes that contain other specific information that may apply to one or more SOPs, for example, lists of reagents or antibodies. The SOP also details Status levels as well as indicating the validation status.

Level of validation of SOPs

We adopted several criteria to classify the extent of validation that had been carried out on any SOP. The level of validation is indicated in the form of a star rating:

- * Bronze indicates validation within *one* Eumorphia laboratory
- ** Bronze and silver indicates validation at *two* different Eumorphia laboratories
- *** Bronze, silver and gold indicates validation at *more than two* Eumorphia laboratories

Working groups – examples of successful and failed validation

We briefly summarise the main areas of development by each working group and additionally we elaborate the data supporting two examples of SOPs that were successfully validated. We also provide one example of a method that had to be extensively adapted due to difficulties with its validation across laboratories.

Results

Animal Handling

The aim here was to establish to what extent animal house regimes differed and to what degree this might impinge upon phenotype outcome, and to identify what standards could be feasibly introduced at this stage. Common maintenance and housing standards were defined including humidity, temperature, light cycle and health screens (adopting FELASA standards). Interestingly, different diets failed to show significant differences in clinical chemistry parameters¹³, but nevertheless we aim in Europe to move to a standardised diet for all centres. One centre had low pH drinking water, but this also failed to impact upon clinical chemistry screens. Importantly, we also standardised the conditions for blood sampling including age, overnight fast, time of sampling and anaesthesia. Interestingly, we observed differences in several clinical chemistry parameters depending upon the method of blood collection (tail vein vs. retro-orbital bleeding, the latter banned in certain European countries) reflecting the problems and complexity of mouse phenotyping.

Clinical Chemistry

With work on the effects of animal handling and housing on clinical chemistry parameters completed, this work group proceeded to define and validate a series of primary tests for clinical chemistry and haematology including ions, metabolites, enzymes and a variety of haematological parameters. Importantly, a mouse plasma pool was distributed amongst the different centres to aid the standardisation of blood biochemistry parameters.

Hormonal and Metabolic

Diabetic and other metabolic diseases represent a major health burden throughout the world. We therefore undertook the development of a battery of tests to explore the hormonal and metabolic status of mouse strains and mutants. The guidelines for animal housing and handling conditions established through the work groups on *Animal Handling* and *Clinical Chemistry* also impact upon the tests developed. Only food intake is classified as a primary test, but in addition 7 Data Generating Primary Extended and Secondary SOPs were developed of which 4 have been validated between different centres – Oral Glucose Tolerance Test (OGTT), Dual energy X-ray absorptiometry (Dexascan), Cold Test and Intraperitoneal Insulin Sensitivity Test (IPIST).

¹³ Champy M-F, Selloum M, Piard L, Zeitler V, Caradec C, Chambon P, Auwerx J (2004) Mouse functional genomics requires standardization of mouse handling and housing conditions. *Mammalian Genome* 15: 768-783

Cardiovascular

Cardiovascular disease is a major cause of morbidity in the human population and a significant risk factor in other disease e.g. diabetes. A schema for primary cardiovascular phenotyping was developed that envisaged two complementary strategies – non-invasive and invasive, the former being used when additional studies are required in longitudinal studies and incorporating electrocardiogram, blood pressure measurement (tail cuff) and echocardiography. The invasive wing of primary phenotyping involves electrocardiogram followed by left ventricular and arterial catheterisation and histology.

Immunology and Infection

Primary immunodeficiency diseases in humans consist of a group of more than 100 diseases with defects in innate and adaptive immune responses, often caused by mutations in genes critical for immune cell development and effector function and homeostatic regulation of the immune system. We developed five primary and primary extended screens to aid the identification of disease models. The primary screens including a nine fluorescence parameter FACs analysis to monitor the presence of the most important immune effector cells, and a new protocol for immunoglobulin isotyping. Three primary extended screens were also developed that permit the characterisation of macrophage functions in mice. Interestingly, validation of the SOPs on inbred strains revealed some surprising differences in some of the immunological parameters and macrophage responses between strains and sex in the course of *Listeria monocytogenes* infection¹⁴.

Renal Function

Renal diseases and renal complications are prevalent and are a significant burden on health-care provision e.g. diabetic nephropathy and idiopathic glomerulonephritis. We have developed a standardised urine collection in metabolic cages as a primary screen. The SOP involves 24 hour urine collection in Tecniplast metabolic cages with free access to food and drinking water with food and water access measured in parallel. Urine and/or plasma biochemical parameters included ions, metabolites (including creatinine) and albumin. Standardisation between centres revealed that normalisation for creatinine excretion provided robust reproducibility for assessment of tubular functions.

¹⁴ Pasche, B., Kalaydjiev, S., Franz, T.J., Kremmer, E., Gailus-Durner, V., Fuchs, H., Hrabé de Angelis, M., Lengeling, A. and Busch, D.H. (2005): „Sex dependent susceptibility pattern to *Listeria monocytogenes* infection is mediated by differential IL-10 production“. *Infect. Immun.* **73**, 5952-5960.

Sensory Function

Sensory defects in hearing and vision account for a very large fraction of human disease syndromes. For example, 1 in every 1000 children are born with hearing impairment of which around half are genetic in origin. We have developed a series of primary and primary extended tests for both auditory/vestibular function and vision. In the case of auditory function, the primary tests for hearing and vestibular impairment (Modified SHIRPA tests including click-box, contact righting, elevated platform and reaching response; acoustic startle response; pre-pulse inhibition and swim test), were closely integrated with the primary tests developed for neurological and behavioural function (see Figure 3 and below). The acoustic brainstem response (ABR) test was adopted and standardised as a Primary Extended test for auditory function. All of the vision tests were primary extended including slit lamp for anterior segment defects and indirect ophthalmoscope or fundus camera for retinal defects. In addition, one functional test for vision, the Optokinetic Response (OKR) test was included and validated as a primary extended SOP¹⁵.

Neurological and Behavioural Function

Neurological and behavioural abnormalities underlie a very broad spectrum of diseases, particularly in the ageing population where neurodegenerative and psychiatric disease is placing an increasing burden on healthcare. We have designed an efficient primary screen for standardised neurological and behavioural phenotyping that incorporates and refines various previous tests. For example, we have included in this battery a modified version of several parameters previously included in the SHIRPA screening protocol. Importantly, as part of the standardisation between centres we have defined a working order and timing for the various tests (see Figure 3).

Figure 4a,b illustrate the validation data between centres for two of the tests (Open Field and Prepulse Inhibition) that are incorporated in the test sequence. These are indicative of test results and demonstrate that, even when using equipment from different sources, strain order for most parameters can be maintained. Additionally, in one test centre where particular substrain substitutions were unavoidable (MRC), strain order was not substantially affected. As expected, however, absolute values varied from centre to centre. Open field parameters, including time spent in the centre of the arena (Figure 4a) were remarkably reproducible across test centres considering the sensitivity of anxiety measures to environment and handling conditions. Only one strain order conflict was evident. The PPI measures were also well-validated considering three separate apparatus were used in test

¹⁵ Jellali A, Meziane H, Ouagazzal A-M, Rousseau S, Romand R, Auwerx J, Sahel J, Chambon P, Picaud S (2005) The optomotor response: A robust first-line visual screening method for mice. *Vision Research* 45: 1439-1446.

centres (Figure 4b). To validate the PPI data, we paid particular attention to differential responsiveness to acoustic stimuli within the test apparatus prior to evaluating the appropriate PPI measurement. For example, a prepulse stimulus of 90 db produced an independent startle response at GSF and MRC but not at CNR and ICS.

In contrast to these successful validations, we initially rejected one test, the rotarod, as a primary SOP (Figure 4c). This test is very commonly used to assess motor function. However, despite our efforts to standardise the first version of the SOP (Rev No 0), the operational procedures between the various centres we found that unacceptable levels of between-centre variation remained, which is in line with a previous attempt in this respect concerning rotarod¹⁶. Such a result emphasises the importance of developing SOPs and the need to examine them rigorously across place and time. We therefore produced a new version (Rev No 1) of the rotarod SOP. This went through the same review processes and subsequently passed validation between laboratories. Key changes were that the apparatus modified adding a foam cover on the rod, the training phase was removed and the test was reduced to three trials instead of four.

Cancer

Due to the variety of tissues and cancer types involved, Cancer Phenotyping is a complex process that requires a multidisciplinary approach involving imaging, pathology, molecular biology and cytogenetics. Moreover, some of the genetically-engineered mice developed as models of human cancer present unique lesions require a careful comparative evaluation with the human disease, as compared to spontaneous or induced tumors in mice. For primary screening we established protocols for tumor description, collection, storage and fixation that should allow both specialized histopathological and molecular phenotyping (e.g. GCH and expression array analysis of tumors). We also established a validated list of primary antibodies for the characterization of mouse tumors by immuno-histochemistry of formalin-fixed tissue. A consortium of mouse and human pathologists within Europe has been formed and has begun to undertake the analysis of specific tumor models in order to reach consensus on unequivocal pathological terms and the corresponding clinical condition as well as to search for genotype-phenotype correlations. A database with virtual slide images of these models is being developed and will be made accessible via the web.

Bone and Cartilage

The skeleton has to fulfil many important functions for the body: stability, carrying the body weight, ensuring mobility and acting as an important store for minerals. There are more than 150

¹⁶ Crabbe JC, Wahlsten D, Dudek BC (1999) Genetics of mouse behaviour: interactions with laboratory environment. *Science* 284:1670-1672

human disorders that reflect skeletal malfunction, including osteoporosis and osteoarthritis. A variety of primary SOPs have been developed including embryological and morphological examinations; blood or urine based indicators for bone metabolism, bone densitometry, X-ray analysis and skeleton preparation.

Gene Expression

Studies of mouse gene expression are an important component of phenotyping. While systematic, genome-wide transcriptional profiling is an important part of molecular phenotyping, other groups have focused on the standardisation of these techniques both in data gathering and data dissemination. However, as an adjunct to pathology analysis and cancer phenotyping, Eumorphia has undertaken the development and validation of SOPs for a number of protocols encompassing the study of individual gene expression in tissue sections.

Imaging

The focus of the work here has not been to develop new SOPs but, firstly, to provide support in validating existing imaging platforms e.g. X-ray analysis and secondly, to undertake development analysis and explore the application of various imaging technologies to mouse phenotyping, including PET, SPECT, MRI and ultrasound. Generic 3D automated algorithms have been developed in order to increase the speed of analysis of images obtained either in vitro or in vivo in embryos or adult animals (for example, analysis of MRI images of cardiovascular structures in mouse models of congenital heart disease). In addition, there has been much focus on the use of X-ray imaging for soft tissue analysis using dedicated contrast agents, including the assessment of intra-venous urography (IVU) for kidney phenotyping.

Pathology

Pathology, the systematic detection and analysis of organ and tissue alterations in adult and ageing mutant mice is a vital component of mouse phenotyping strategies. We have devised a primary histopathological screen in order to detect the broadest array of tissue abnormalities in a mouse and to correlate them with underlying mutations. The protocol recommends the analysis of groups of 24 mice (equal numbers of male and female and of mutant and wild types) that are divided into two sub-groups. The first, smallest sub-group consisting of mutant mice is subjected to a systematic high-throughput histological analysis of hematoxylin and eosin-stained sections of each of 40 organs. The second and largest group comprising the remaining mice and the majority of the control mice are subject to a systematic necropsy and to a targeted histological screen. Mutant organs in which

morphological defects are diagnosed are then forwarded to a primary extended assays involving the analysis of organ volumes, the use of organ and pathology-specific strains and/or the detection of cell proliferation and cell death.

Respiratory Function

The basis of the first-line respiratory exploration is to detect the principal respiratory disorder associated with the most widely occurring chronic inflammatory respiratory diseases affecting patients in Europe: asthma and allergy, chronic obstructive pulmonary disease (COPD), and lung fibrosis. These diseases are associated with bronchial hyper-responsiveness. The first-line screen documents the airway responsiveness in mice. SOPs are included for an asthma model, COPD model and airway inflammation

Case studies from the systematic application of standardised screens

The application of the EMPReSS SOP in a systematic manner to background and mutant strains and both sexes has already uncovered several interesting new models for disease. This approach is different to the usual hypothesis-lead science and, in the case of background strains, has yielded new models that otherwise may not have been recognised.

Cardiovascular exploration

- There has been extensive evaluation of the SOPs to compare invasive and non-invasive measurements. For blood pressure and heart rate these give comparable results.
- An interesting problem of cardiac function has been identified in the C3HeB/FeJ strain.
- Measurement of plasma atrial natriuretic peptide (ANP) has been developed as a tool to identify left ventricular dysfunctions and hypertrophy.

Immunology and infectious diseases

- A two-centre cross validation of FACS analysis of peripheral blood cells and determination of immunoglobulins in mouse sera was performed. Good correlation was achieved in the results.
- An interesting increase in the frequency of CD19⁺ B cells in C57BL/6J mice as compared to the other inbred strains investigated.
- A luminex bead system is being developed to measure up to 100 serum parameters in a single measurements in a bead array flow cytometer with a view to instigating this as a high-throughput screen.

Renal

- A standardised protocol for metabolic cages with associated sampling and analysis of blood and urine has been validated in 2 centres. The parameters measured were in the same range at both institutes.
- 129S2/SvPas mice (males) were found to differ from the other background strains tested in that they had a higher urinary excretion of electrolytes (Na, K, Cl, Ca). This was mainly linked to a higher glomerular filtration rate in young mice and to changes in tubular handling in older mice.
- Renal imaging is being developed in conjunction with Animage.

Sensory systems

- Data from the cross-validation of the optokinetic response test using a number of background and mutant strains in 2 laboratories has identified absent or poor vision in C3HeB/FeJ, BALB/cByJ mice and several novel atypical retinal degeneration (*atrd*) mutants of *Pde6b*.
- The test allows the progressive loss of vision, concomitant with loss of photoreceptors, to be tracked and quantified. This work on *atrd* mutants has been published¹⁷.

Informatics: the EMPReSS SOP and EuroPhenome databases

To fully realise the potential of model organisms to bridge the gap between phenotype and genotype it is essential to provide structured descriptions of phenotypes that can be interpreted in a consistent fashion. We have recently suggested ways of using combinations of ontologies to describe mouse phenotypes^{18,19} and provided tools that allow the storage, active updating and visualisation of multiple ontologies²⁰. Central to this approach is the establishment of validated SOPs for the measurement of phenotypic attributes. We believe the screen or assay used to ascertain phenotype governs the phenotype that will be detected and therefore must play a central role in phenotype representation. We are in the process of constructing the controlled vocabularies of assays required for implementation of this schema. From this perspective the EMPReSS resource represents a collection of well-defined assays that can be linked directly to phenotype data.

¹⁷ Hart, A.W., McKie, L., Morgan, J.E., Gautie, P., West, K., Jackson, I.J. and Cross, S.H. (2005) Genotype-phenotype correlation for mouse *Pde6b* mutations. *Invest Ophth Vis Sci* 46: 3443-3450.

¹⁸ Gkoutos et al (2004). Building mouse phenotype ontologies. *Pac. Symp. Biocomput.* 178-189

¹⁹ Gkoutos GV, Green ECJ, Mallon AM, Hancock JM, Davidson D. (2005) Using ontologies to describe mouse phenotypes. *Genome Biol.* 6: R8

²⁰ Gkoutos et al (2005). CRAVE: a database, middleware and visualisation system for phenotype ontologies. *Bioinformatics.* 21, 1257-1262

To facilitate the storage and processing of the SOPs we have created SOPML, an XML language that allows the description of SOPs. The SOPs generated by different institutes are automatically annotated using a SOP template and stored in SOPdb, the underlying database. The documents produced by this process are automatically validated against the XML schema and manually curated by a domain expert before being committed in the database. The combination of XML-based markup, RDF document metadata and XSLT transforms facilitates filtering, advanced indexing, searching and rendering of the information²¹. The EMPReSS SOP database is accessible at <http://empress.har.mrc.ac.uk>²².

We have also developed a phenome database for Eumorphia, *EuroPhenome*, which holds the baseline data from our standardisation and validation experiments on inbred strains. The EuroPhenome database links phenome data to the EMPReSS SOPs and is available through www.europhenome.eu.

Conclusions

The challenge for mouse phenotyping is to develop a robust set of protocols accessible to and utilisable by laboratories and centres both large and small. The Eumorphia consortium has brought together a large and diverse group of geneticists and physiologists to develop and assess mouse phenotyping tests in response to this challenge recognising that a collective approach underpinned by appropriate scrutiny and validation will deliver robust and workable phenotyping platforms. Eumorphia has established a new set of phenotyping methodologies for the functional annotation of the mouse genome, comprising a database of validated SOPs covering most of the major mammalian body systems. The value of EMPReSS lies in its ability to deliver phenotype outcomes that are comparable between laboratories and over time. In addition, the EMPReSS database has been constructed so that it is integrated with the data and ontological structures that are required to systematically represent, store and search mouse phenotype data. EMPReSS will continue to develop in a number of ways. Firstly, the application of EMPReSS in large scale screens will allow us to further refine and validate the current SOPs. Secondly, it will be important to expand the repertoire of phenotype tests that are available, including primary as well as secondary and tertiary screens. We plan to employ EMPReSS as we begin the process of phenotyping the large numbers of mouse mutants generated through the planned large-scale mouse mutagenesis programmes.

²¹ Gkoutos et al (2001). Chemical markup, XML, and the world-wide web. III. Toward a signed semantic chemical web of trust. *J. Chem. Inf. Comp. Sci.* 41: 1124-1130

²² Green ECJ, Gkoutos GV, Lad HV, Blake A, Weekes J, Hancock JM (2005) EMPReSS: European mouse phenotyping resource for standardized screens. *Bioinformatics.* 21:2930-2931

Networking

One of the key successes of Eumorphia was that it brought together scientists from a wide variety of disciplines. Scientific exchange was encouraged at three kinds of meetings:

- annual project assembly (~70 participants) where scientists working on the projects came together to present progress and plan for the year ahead. This also allowed scientists, working within different workpackages of the project, to learn about the work of other workpackages
- workpackage meetings (~10-30 participants), which were working meetings focused on one research area or several areas, to include Eumorphia scientists and invited external experts in the area
- annual open meeting (~150 participants); a large 2 day scientific conference with invited external speakers, internal speakers and a poster session. This meeting was open to all Eumorphia scientists, invited external scientists and external participants on approval of an abstract of their work

The smaller meetings allowed for monitoring and planning of the project and the larger open meetings allowed advertisement of the project to a wider audience. Junior scientists were encouraged to play a large part in the project, by presenting at the annual assembly and workpackage meetings and also by presenting posters to a wider scientific audience at the annual open meetings. As a result of these meetings, new collaborations were formed, for example between the biologists and experts in small animal imaging. These collaborations were continued within the project and also formed the basis for new collaborative projects.

Eumorphia Meetings

Title	Date	Location
Cardiovascular Symposium Open meeting hosted by WP5	24 February 2006	Barcelona, Spain
3 rd Annual Project Meeting (open meeting)	23-24 February 2006	Barcelona, Spain
WP9 & 10: PNS & skeletal muscle Behaviour & cognition	29 November 2005	Rome, Italy
WP11: Cancer Pathology Expert Panel Meeting	19-20 November 2005	Amsterdam, The Netherlands
WP8: Sensory Systems	17 November 2005	Edinburgh, UK
Biology of the Immune Defence Open meeting hosted by WP6	1-2 November 2005	Braunschweig, Germany
EuroMouse Open meeting hosted in conjunction with PRIME	14-15 October 2005	Venice, Italy

Title	Date	Location
WP7 Renal	5 September 2005	Lyon, France
3 rd General Assembly: Internal meeting	16-17 March 2005	Madrid, Spain
WP3 & 4: Clinical chemistry Hormonal & metabolic	24 January 2005	Strasbourg, France
WP8, 9, 10 & 14: Sensory systems, PNS & skeletal muscle Behaviour & cognition and Imaging	9-10 December 2004	Rome, Italy
WP8: Vision	24-25 November 2004	Edinburgh, UK
2 nd Annual Project Meeting (open meeting)	5-7 October 2004	Heathrow, UK
Mouse Mutagenesis Meeting: Open meeting hosted by WP16	3-4 October 2004	Heathrow, UK
WP14 & 17: Imaging and Bioinformatics	14 September 2004	Edinburgh, UK
WP11 Cancer Phenotyping	5-6 July 2004	Madrid, Spain
WP9 & 10: PNS & skeletal muscle Behaviour & cognition	3 July 2004	Lyon, France
WP9, 10 & 14: PNS & skeletal muscle Behaviour & cognition and Imaging	2 July 2004	Lyon, France
WP7: Renal	14 May 2004	Strasbourg, France
WP9 & 10: PNS & skeletal muscle Behaviour & cognition	23 April 2004	Rome, Italy
2 nd General Assembly: Internal meeting	18-20 March 2004	Munich, Germany
WP16: Mutagenesis	10 March 2004	Cambridge, UK
WP5: Cardiovascular	2 March 2004	Manchester, UK
WP15: Pathology	20 February 2004	Strasbourg, France
WP13: Gene Expression	6 February 2004	Rome, Italy
WP8: Sensory Systems	2 February 2004	Harwell, UK
WP5: Cardiovascular	13 January 2004	Paris, France
WP9 & 10: PNS & skeletal muscle Behaviour & cognition	15 December 2003	Rome, Italy
1 st Annual Project Meeting (open meeting)	8 October 2003	London, UK
Phenotype screens for mice: Developing an integrated platform.	6-7 October 2003	London, UK
WP5 & 14: Cardiovascular Phenotyping & Imaging	17 September 2003	Heathrow, UK
WP9 & 10: PNS & skeletal muscle Behaviour & cognition	11 July 2003	Rome, Italy
WP8: Sensory	8 July 2003	Harwell, UK
WP11: Cancer Phenotyping	4 July 2003	Amsterdam, The Netherlands

Title	Date	Location
WP16: Mutagenesis	23-28 June 2003	Paris, France
WP15: Pathology	23 June 2003	Strasbourg, France
WP1, 3 & 4: Animal handling Clinical chemistry Hormonal & metabolic	13 May 2003	Strasbourg, France
1 st General Assembly: Internal meeting	15-17 January 2003	Strasbourg, France
WP14:Imaging	12 November 2002	Lyon, France

Training

Eumorphia trained a new cadre of young scientist who were employed by the project. These scientists were given the opportunity to learn new skills by going on working visits to other Eumorphia laboratories under Eumorphia sponsorship. In addition, Eumorphia ran a number of training courses that were open to Eumorphia and external scientists.

Training courses

Title	Date	Location
Embryo Handling and Cryopreservation of the Mouse	January 2006	Harwell, UK
Practical Course on Mouse Histopathology	December 2005	Strasbourg, France
Medical Imaging for Small Animals	December 2005	Lyon, France
An Introduction to Pathology: Mouse Models of Human Cancer	November 2005	Amsterdam, The Netherlands
Eumorphia & EMBO Summer School	September 2005	Bischoffheim, France
Embryo Handling and Cryopreservation of the Mouse	May 2005	Harwell, UK
Behavioural Phenotyping	April 2005	Strasbourg, France
Embryo Handling and Cryopreservation of the Mouse	October 2004	Harwell, UK
Medical Imaging for Small Animals	June 2004	Lyon, France
Shirpa Training	September 2003	Harwell, UK

Exploitation and dissemination of results

The database of primary and primary extended SOPs is now available for all scientists to use. It is important for us to continue to advertise its existence and promote its use amongst research groups and pharmaceutical companies. We must adopt and utilise standardised

robust phenotyping platforms, where phenotyping is performed according to standardised procedures, in order to generate extensive and comparable phenome datasets that will allow mouse geneticists to share and compare phenotype data.

A key focus has been to bring EMPReSS to the attention of the wider community. We have promoted Eumorphia and EMPReSS through scientific publications and in presentations and posters at academic conferences and in invited talks at research institutions. Members of the consortium also published a Correspondence in *Nature Genetics*²³ which explained the importance of standardised phenotyping and the development and validation of the EMPReSS SOPs. The Eumorphia consortium also produced a simple and visual leaflet explaining Eumorphia and EMPReSS. This has been distributed widely, e.g. at academic conferences, to members of other EU consortia, to politicians and policy makers and industry. We have also written several articles for the EUROPA Fundamental Genomics Website, which is coordinated by the European Commission. A book dedicated to mouse phenotyping has also been published²⁴ many of the Eumorphia partners and workpackage contributed chapters to this book.

It is important to introduce the work of the Eumorphia consortium and the use of standardised phenotyping to the wider scientific and pharmaceutical communities. To this end, the three Eumorphia annual meetings were open for scientists external to Eumorphia to attend. This also helped to raise the scientific profile of Eumorphia by presenting results from the application of the standardised tests. It also created new scientific collaborations that have strengthened EMPReSS.

A key potential user of EMPReSS and potential advocate of it is the pharmaceutical and biotechnology sector. To promote the uptake of EMPReSS by industry, a meeting was held to introduce Eumorphia and EMPReSS and to learn how EMPReSS may be put to use by companies. The meeting was an open forum to discuss how Eumorphia can help industry and vice-versa, and how we might coordinate efforts to our mutual benefit. Representatives from Artemis Pharmaceuticals, AstraZeneca, Charles River Laboratories, GlaxoSmithKline, Merck and Novartis were present. They were very positive about EMPReSS and the proposed high throughput of EMPReSSslim. This working group will meet again to discuss EMPReSSslim and the application of EMPReSS SOPs within their research and phenotyping services.

²³ Brown, S.D.M., Chambon, P., Hrabé de Angelis, M. and The EUMORPHIA Consortium (2005). EMPReSS: standardized phenotype screens for functional annotation of the mouse genome. *Nature Genetics*, 37: 1155.

²⁴ Standards of Mouse Model Phenotyping. Eds. Hrabé de Angelis, M., Chambon, P., Brown, S. (2006) Wiley-VCH, Weinheim, (ISBN 3-527-31031-2).

The phenome database is a valuable resource of phenotype data on background strains. A large-scale validation is outside of the scope of the Eumorphia project. However, future research projects could build on this and add data for background strains and mutant strains. It is important to build links with other phenome databases and we are working with colleagues in USA, Australia and Japan to coordinate and link SOPs and phenome databases. To this end, we held an international mouse phenome workshop in February 2006 to discuss ways to integrate the various resources that store mouse phenome information. As a result of this, we are piloting several actions to add a search facility to pull information out of 3 existing databases (JAX MGD, Eumorphia phenome database).

With large-scale projects such as EUCOMM to generate mutations in 20,000 genes in mouse embryonic stem cells, the need for high-throughput phenotyping is clear. To this end we have developed EMPReSSslim, a subset of EMPReSS SOPs, that can be used to thoroughly screen one mouse line. We have formed a consortium of mouse clinics which can perform the primary high-throughput phenotyping in EMPReSSslim and secondary phenotyping centres to perform more in depth investigations. We are seeking EC and national support for this.

Policy related benefits

Eumorphia has contributed towards the aims and policies of the FP5 “Quality of Life” Programme. It was also designed to look forward to the aims and policies of the FP6 Programme “Life Sciences, Genomics and Biotechnology for Health”. The original proposal was set up with 4 strategic goals to address these policies:

Scientific goal: to develop the mouse as a tool to characterize gene function

Our aim was to build upon the unique insight that the mouse can bring to many physiological and biochemical pathways underlying human diseases. Use of standardised phenotyping protocols in EMPReSS will facilitate the sharing and comparison of data on gene function and disease models. They will also form the foundation for a high-throughput screening programme to determine the phenotype of large numbers of mutants. The EuroPhenome database will store this information and allow access by researchers worldwide. The ultimate aim is to determine the role of each gene in physiological and biochemical pathways.

Medical goal: to develop mouse models to understand human diseases

Human disease conditions are often very complex due to disturbed interdependencies inside the human body. Information about these dependences can be obtained using mouse models. The

generation and phenotyping of mouse models allows the assessment of the contribution of specific genes to disease, as well as the study of gene-gene and gene-environment interactions in disease. The breadth and depth of the screens included in EMPReSSslim will give information on a wide spectrum of physiological and disease areas for each strain.

Economic goal: to implement the use of the mouse to develop new therapeutics by pharmaceutical and biotechnology industries

Biopharm and biotech industries increasingly recognize the importance of the mouse for the identification of new targets. In addition, many of the commercial mouse breeders are beginning to offer phenotyping services. We have set up an industry working group to present EMPReSS to them and discuss their interest in phenotyping and ways for them to use EMPReSS SOPs.

Educational and federative goals: to leverage Europe's lead in many areas of mouse biology

Eumorphia has produced a new cadre of trained mouse geneticists through the training posts that were sponsored in the project. In addition to the training that they received in their host institutions, they were able to travel to other laboratories within the Eumorphia consortium, on funded working visits, to learn new techniques and ensure that they were performing SOPs and the analysis of results in a standard way. In addition to this, Eumorphia sponsored 8 training courses in areas such as: cryopreservation of sperm and embryo and embryo transfer, histology, pathology, imaging and behavioural tests. Eumorphia also set up and ran a residential, 5-day summer school. Experts in the field of mouse functional genomics from around the world gave lectures to the students. The Eumorphia courses and summer school were open to scientists outside Eumorphia.

Benefits of an EU-wide program

The establishment of a comprehensive mouse phenotyping program and the training of mouse pathologists was made possible through the collaboration of all of the partners within the consortium. As envisaged in the original proposal for Eumorphia, this was achieved through the following areas:

Creation of a critical mass through collaboration and cooperation

EMPReSS was created through a multi-centre and multi-disciplinary effort. There was tremendous cross-fertilisation between institutes in individual workpackages and between workpackages.

Standardisation to compare results and avoid duplication

EMPreSS is the first large-scale, mouse phenome program to bring together standardised phenotyping procedures. This is important, as we need to standardise our approaches to phenotyping; if mouse genetic centres around the world use various environmental conditions, or adopt quite different test procedures, then much of the ensuing datasets will not be comparable. Reduction of duplication will reduce the numbers of animals used in the research. The SOPs are a refinement of protocols; animal welfare was considered during this refinement process

Improving the social acceptance of the mouse as a tool for medical research

In Eumorphia, we have made efforts to inform the public on the benefits of mouse functional genomics research and its relevance to human health. We have a public website (www.eumorphia.org) and an information leaflet. We have tried to hold open meetings with members of the public and/or representatives from charities and patient organisations. It has been surprisingly difficult to attract people to these meetings. We are still continuing these efforts.

Education of integrative biologists, a European duty

As described above, there was a big effort in Eumorphia to train young scientists within the consortium, as well as external scientists interested or involved in the field.

Contribution to Community social objectives

As outlined in the original proposal for the Eumorphia project, the development of EMPReSS has made a number of key contributions to the EU social objectives, namely:

1. It has delivered new mouse disease models and has contributed to the identification of underlying genes and genetic pathways involved with the disease process. Several new models have been discovered through implementing EMPReSS protocols on background and mutant strains.
2. Application of EMPReSS protocols will improve safety. In toxicological studies and toxicity testing, it will allow a more robust and comprehensive assessment of phenotype that is standardised across all laboratories. It will also allow a standardised documentation of the physiological and pathological phenotypic effects of toxins.
3. It provided opportunities for training and integration of mouse functional genomics with veterinary, medical skills as well as bioinformatics.
4. The networking element of eumorphia allowed experts, within and outside Eumorphia, to gather in groups to develop and standardise the SOPs. It also formed new

collaborations between Eumorphia partners that are being taken forward in other projects. Partnerships were also formed across disciplines, for example, the experts in imaging were introduced to biologists who could provide the mouse models.

5. Eumorphia attempted to engage with lay people/groups.
6. The improvements in the characterisation of diverse phenotypes in the mouse will allow a reduction in the number of mice, as well as larger animals, for the study of disease or basic biochemical and physiological processes.

In summary, Eumorphia was successful, as it brought together a critical mass of scientists from diverse fields who worked together in groups, as well as forming new partnerships across groups. It has enabled Europe to take a lead in the establishment of high-throughput, phenotyping using standardised SOPs. We are now in a position to apply this high-throughput phenotyping to mouse models being developed in large-scale European mutagenesis programmes (such as EUCOMM, the European Conditional Mouse Mutant program). In addition, Eumorphia has produced a new cadre of scientists trained in mouse functional genomics who can continue with the lead that Eumorphia has taken.

Eumorphia publications

WP1: Animal handling and housing

Argmann, C.A., Auwerx, J. Minimizing Variation Due to Genotype and Environment. In *Current Protocols in Molecular Biology*. Eds. Assubel, F., Brent, R., Kingston, R.E., Moore, D.M., Seidman, J.G., Smith, J.A., Struhl, K. John Wiley & Sons, 2006, pg. 29A.2.1-29A2.3

Argmann, C.A., Chambon, P. and Auwerx J. (2005) Mouse phenogenomics: the fast track to 'systems' metabolism. *Cell Metabolism* 2, 349-360.

Argmann, C.A., Dierich, A. and Auwerx, J. General considerations in mouse phenotyping. In *Current Protocols in Molecular Biology*. Eds. Assubel, F., Brent, R., Kingston, R.E., Moore, D.M., Seidman, J.G., Smith, J.A., Struhl, K. John Wiley & Sons, 2006, pg. 29A.1.1-29A2.2

Champy, M.-F., Selloum, M., Piard, L., Zeitler, V., Caradec, C., Chambon, P. and Auwerx, J. (2004) Mouse functional genomics requires standardization of mouse handling and housing conditions. *Mammalian Genome* 15, 768-783.

WP2: First-line phenotyping

Standards of Mouse Model Phenotyping. Eds. Hrabé de Angelis, M., Chambon, P., Brown, S. (2006) Wiley-VCH, Weinheim, (ISBN 3-527-31031-2).

Brown, S.D.M., Chambon, P., Hrabé de Angelis, M. and The EUMORPHIA Consortium (2005). EMPReSS: standardized phenotype screens for functional annotation of the mouse genome. *Nature Genetics*, 37: 1155.

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Figures and tables

Table 1. Eumorphia workgroups

Clinical Chemistry & Haematology
Hormonal & Metabolic Systems
Cardiovascular
Allergy & Infectious Diseases
Renal
Sensory systems (Vision & Auditory)
Peripheral/central nervous system & muscle
Behaviour, Cognition & neurological
Cancer Phenotyping
Bone & Cartilage
Gene Expression
Imaging
Necropsy, Histology & Pathology
Respiratory

Table 2. Summary Table of SOPs

(* SOPs validated ready for inclusion in the release of the new version of EMPReSS)

Clinical chemistry and haematology

Differential blood count
Blood Collection by retro-orbital puncture
Blood Collection by intra-cardiac puncture
Blood sample handling-Clinical chemistry
Blood sample handling-Haematology
Blood sample handling-Coagulation
Clinical chemistry parameters
Haematology tests
Gas anaesthesia
Coagulation tests
Annex 1: Reagents for blood clinical chemistry on AU400
Annex 2: Calibrators for clinical chemistry on AU400
Annex 3: Controls for clinical chemistry on AU400

Hormonal and Metabolic Systems

Simplified metabolic cages
Metabolic cages
Dexa-scan analysis
Simplified Intra-Peritoneal Glucose Tolerance Test (I.P.G.T.T)
Intra-Peritoneal Glucose Tolerance Test (I.P.G.T.T)
Cold test
Meal Tolerance Test (M.T.T)
Oral Glucose Tolerance Test (O.G.T.T)

Cardiovascular

Non invasive blood pressure and heart rate
Invasive blood pressure
Invasive left ventricular haemodynamics
Blood pressure by Telemetry
Surface electrocardiography (ECG)
Echocardiography (ECG)

Allergy and Infectious diseases

Titration of antibody solutions for FACS analysis
Cross linking phycoerythrin to antibodies
Coupling fluorescein to antibodies
FACS analysis of peripheral blood cells
Determination of Immunoglobulin concentrations in the serum of mice
Isolation of murine bone marrow derived macrophages
Isolation and culturing of proteose peptone elicited peritoneal macrophages
Isolation and culturing of thioglycolat elicited peritoneal macrophages
Quantification of TNF α production by PAMP stimulated macrophages
Infection of proteose peptone macrophages with *Listeria monocytogenes*
Quantitative measurement of iNOS activity after macrophage stimulation
Staining protocols for *Listeria monocytogenes* infected macrophages
Counting cells using a Thoma chamber
Annex: Characterisation of macrophage functions
Inoculation of *Listeria monocytogenes* EGD for infection *
Infection of mice with *Listeria monocytogenes* EGD *
Tissue monitoring (*Listeria monocytogenes*) *
Inoculation of *Streptococcus pyogenes* A20 for infection *
Infection of mice with *Streptococcus pyogenes* A20 *
Blood monitoring (*Streptococcus pyogenes* A20) *
Health monitoring of mice in infection experiments *

Sensory Systems

Vision

Optokinetic response test
Fundus and Angiography
Using an indirect ophthalmoscope
Using a slit lamp

Auditory and Vestibular

Modified SHIRPA protocol specific to sensory systems
Elevated platform and reaching response test
Acoustic startle and pre pulse inhibition
Swim ability test
Auditory Brainstem Response (ABR)

Behaviour and cognition

Open Field
Modified SHIRPA
Grip Strength
Y-maze
Acoustic startle and pre pulse inhibition
Tail Flick
Tail suspension
Swim ability test
Rotarod test

Cancer Phenotyping

The macroscopic description of a tumor process
Freezing of murine tumor tissue
Fixation and processing of murine tumor tissues enabling diagnostic, immunohistochemical and molecular analysis
Annex 1: Staining with Harrison's fixative
Annex 2: Optimization of specificity and sensitivity in immunohistochemical investigations on formalin fixed, paraffin-embedded murine (tumor) tissue
Immunostaining using streptavidin-biotin-horseradish peroxidase enhancement
Manual production of Tissue Micro Arrays
Isolation of DNA from frozen tissue, immunohistochemical and molecular analysis
Isolation of DNA from formalin-fixed, paraffin-embedded tissue
Isolation of total RNA from frozen tissue
Monitoring the quality of total RNA by agarose gel electrophoresis
Reverse transcription of mRNA. First strand cDNA synthesis
Isolation of total RNA from formalin-fixed, paraffin-embedded tissue

Bone, Cartilage, Arthritis, Osteoporosis

Dysmorphology
Ionic fraction of Ca²⁺ in whole blood
Bone densitometry
X-ray
Micro CT Imaging
Skeletal preparation of a mouse

Gene Expression

In vitro transcription of digoxigenin-labelled riboprobes
In situ hybridisation of cryosections with digoxigenin labelled probes
Whole-mount *in situ* hybridisation of mouse embryos
Automated whole-mount *in situ* hybridisation of mouse embryos
In vitro transcription of 35S-labelled riboprobes
In situ hybridisation of cryosections with 35S labelled probes
Annex 1: *In situ* hybridisation Optimisation of specificity and sensitivity-troubleshooting notes
Annex 2: *In situ* hybridisation Working with RNA

Necropsy Examination, Pathology, Histology

Annex 1: A systematic histopathology flow scheme

First line phenotyping necropsy

Trimming fixed tissues from necropsy

Fixation

Tissue fixation with Bouins solution

Tissue fixation with Davidsons solution

Tissue fixation with 10% buffered neutral formalin

Tissue fixation with gluteraldehyde

Tissue fixation with 4% buffered paraformaldehyde

Tissue fixation by perfusion

Tissue fixation with periodate-lysine-2% paraformaldehyde (PLP)

Embedding

Demineralsation of long bones using EDTA

Fixation decalcification processing and paraffin embedding of whole adult mouse head

Freezing tissues for histopathological analyses

Tissue processing and embedding in paraffin

Sectioning

Sectioning from paraffin embedded tissues

Cryosectioning

Proliferation Apoptosis

Immunodetection of the cell proliferation marker Ki67

Detection of 5-bromo-2-deoxyuridine (BrdU) incorporation

Immunodetection of cells in mitosis

In situ detection of cell death

Staining

Haematoxylin and eosin staining of histological sections

Modified Mallory's trichrome staining of histological sections

Luxol fast blue and cresyl violet staining of brain and spinal cord

Orcein staining for elastic fibres

Von Koss's silver nitrate method for detection of calcified tissue deposits

Periodic Acid Schiff (PAS) staining of histological sections

Oil red O staining of histological sections

Detection of cholesterol esters in histological stains

Mammary gland whole mount preparation

Alizarin red and alcian blue method for staining bones and cartilage in the adult mouse

Sirius red staining for collagen

Congo red for amyloidosis

Oral

Phenotyping mouse oral cavity - primary first line

Phenotyping mouse oral cavity - primary extended

Figure 1. Development of SOPs and the validation process

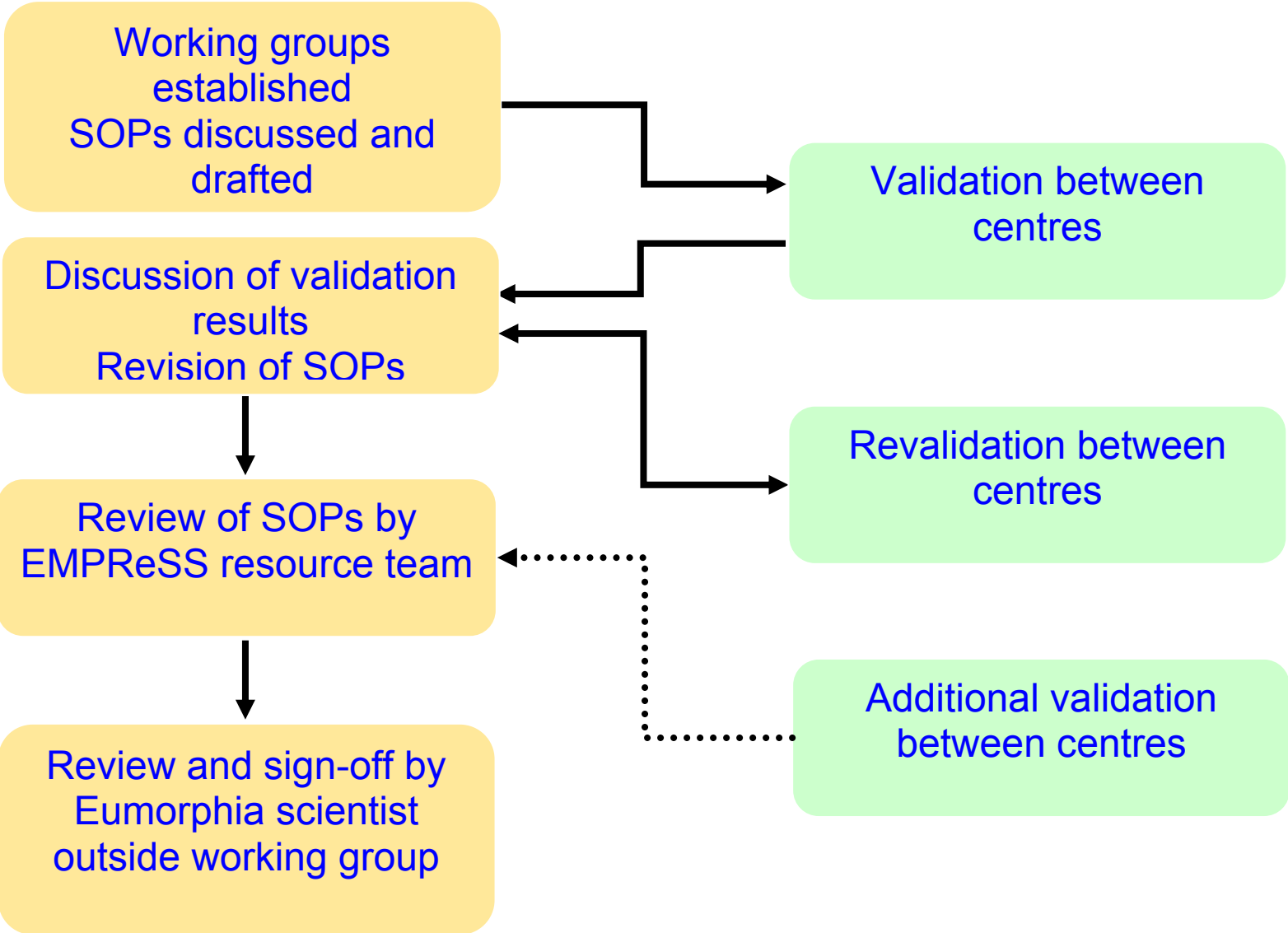


Figure 2. EMPreSS SOP format



Standard Operating Procedure Title: **Open Field**
Doc. Number: 10_001
Rev No: 0
Status: 3
Validation: ★★
Date Issued: 01/06/04 : Type: 1F

Table of Contents

1. Purpose
2. Scope
3. Safety Requirements
4. Associated Documents
5. Notes
6. Quality Control
7. Equipment
8. Supplies
9. Procedure
10. Supporting Information
11. History Review

Figure 3. Order and timing of primary neurological, behavioural and sensory tests in the EMPReSS protocol

The diagram summarises the order defined for the primary neurological and behavioural tests adopted and validated within EMPReSS. Various sensory screens are integrated within the testing sequence to provide an integrated set of tests covering all domains. Note that the Rotarod test was poorly validated so the original SOP was rejected as part of the primary EMPReSS screen (see text).

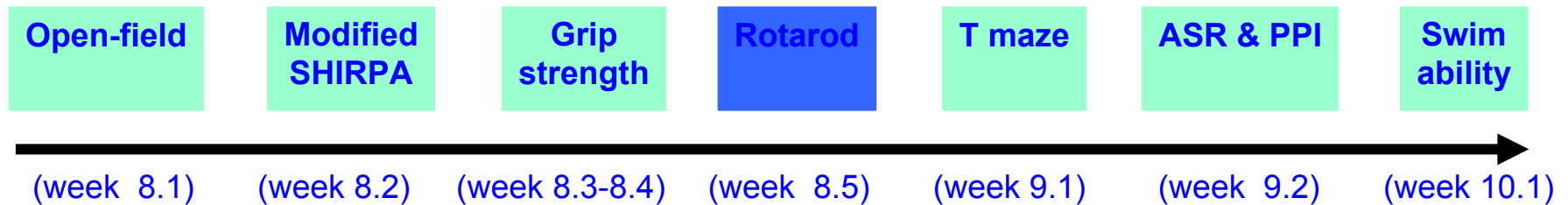
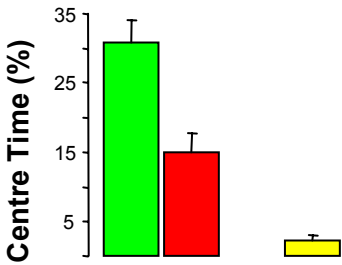
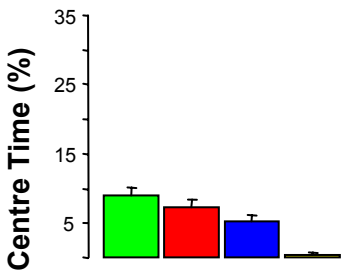
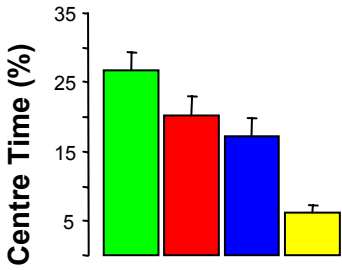
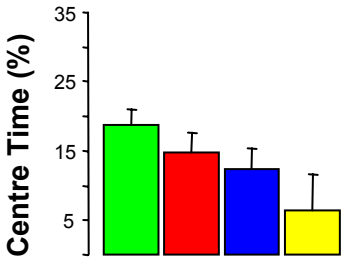
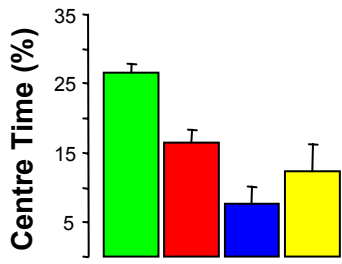


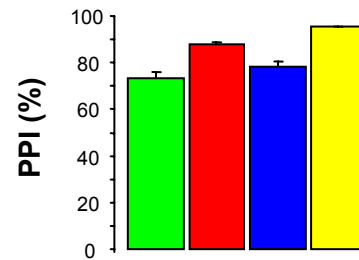
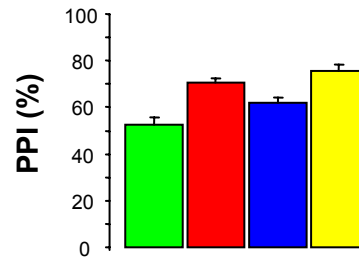
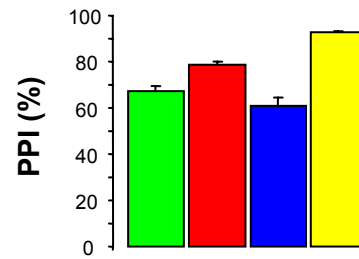
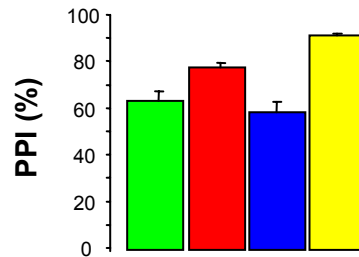
Figure 4. Validation of primary behavioural screens across test centres

A comparison of Open Field Centre Time, Prepulse Inhibition of Acoustic Startle Response (PPI) and Rotarod performance across five test centres (CNR, GSF, MRC, ICS and EMBL) and in four mouse inbred strains (C57 ■, C3H ■, BALB ■ and 129 ■). For each test parameter mean values and standard errors are plotted (n=10-12 males per group). In most cases, test results could be validated across laboratories even when different test equipment was used and where particular substrains were substituted. **a).** Open Field Centre Time. Percentage time spent in the centre of the arena is indicated. Quantitative differences in this parameter were seen across all test centres, presumably due to the fact that different apparatus and measurement systems were used. Remarkably, however, strain order was conserved in four out of five test centres with C57>C3H>BALB>129. In one test centre (CNR), the strain order of BALB and 129 was reversed indicating that the emotionality of one or both of these strains may be particularly sensitive to environment or test conditions. BALB performance could not be evaluated at EMBL. **b).** PPI. Percentage inhibition of acoustic startle response is indicated. To calculate an appropriate measurement of PPI for the apparatus at each test centre, the maximum prepulse intensity that did not produce an independent startle response in mice was used to calculate the PPI value. Thus, an 80 db stimulus was used as prepulse in GSF and MRC, while the prepulse intensity in CNR and ICS was 90dB. Again some quantitative differences in this parameter are evident although strain order is essentially conserved across all test centers with 129>C3H>C57>BALB. PPI was not assessed at EMBL. **c).** Rotarod. The mean latency (in seconds over 4 trials) to fall from an accelerating rotarod is indicated. C57 performance could not be evaluated at MRC and BALB performance could not be evaluated at EMBL. Large quantitative differences in this parameter were observed and no consistent strain order could be established. Lack of validation for this test was credited to the different apparatus used (diameter and texture of the rotating rod) and to the differential assessment of strain performances. These issues are being attended to the production of a new version of this test.

a)



b)



CNR

GSF

MRC

ICS

EMBL