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Behavioural battery testing: Evaluation and behavioural outcomes in 8 inbred mouse strains

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ABSTRACT

The use of large scale behavioural batteries for the discovery of novel genes underlying behavioural variation has considerable potential. Building a broad behavioural profile serves to better understand the complex interplay of overlapping genetic factors contributing to various paradigms, underpinning a systems biology approach. We devised a battery of tests to dissect and characterise the genetic bases of behavioural phenotypes, but firstly undertook to evaluate several aspects considered potentially confounding for mapping quantitative traits. These included investigating: individual versus sibling housing; testing at different times during the day; battery versus non-battery testing; and initial placement within the light–dark box. Furthermore, we assessed how behavioural profiles differed in our battery across 8 inbred strains. Overall, we found the behavioural battery was most sensitive to paired-housing effects, where weight and some measures in the open field, elevated plus maze and light–dark box differed significantly between sibling housed and singly housed mice. Few large effects were found for testing at different times of day and battery versus non-battery testing. Placement in the light–dark box influenced activity and duration measures, which profoundly affected the analysis outcome. Behavioural profiles across eight inbred strains (C57BL/6J, 129S1/SvImJ, A/J, BALB/cByJ, C3H/HeJ, DBA/2J, FVB/NJ, and SJL/J) demonstrated some robust strain ranking differences for measures in the open field and light–dark tests in our battery. However, some tests such as the elevated plus maze produced incongruous strain ranking effects across measures. The findings reported herein bear out the promise of behavioural batteries for mapping naturally occurring variation in mouse reference populations.

1. Introduction

A major difficulty in using mouse models to map QTLs for complex traits, such as behaviour, is the large number of animals required for the undertaking to be successful. This complication is further compounded by the paradox of dissecting complex traits into manageable components without losing sight of the multitude of underlying factors that may be interacting and attributable for the trait in question. Behavioural phenotyping assays performed in isolation may oversimplify and fail to account for the complex networks that are involved, so multi-scale phenotyping approaches are desirable as they provide much more information on many levels and enable us to generate a comprehensive profile of a phenotype [1–3]. Studies that aim to dissect and map behaviours using these approaches have become popular in the post-genomic era [4–8]. While comparisons between behavioural battery tested and naïve mice have been reported to demonstrate task-dependent differences, in the main behavioural profiles are found to be comparable [9]. In battery testing we can make use of a composite of measures across multiple tests and look for their correlation with overlapping behavioural phenotypes, which can in turn be complemented by association with biological markers to gain a broad appraisal of the underlying mechanisms [10–12]. A further advantage is that since screening can be performed using the same animals throughout a carefully devised phenotyping platform, these studies can actually serve to reduce the number of animals needed to effectively map QTLs for behaviours.

Mapping studies that make use of a battery of tests can only be fruitful if the experimental design is given careful consideration. Homogeneous test groups that account for age and weight across animals are an important starting point. However, standardising environmental variables in particular, which mouse behavioural batteries may be sensitive to, such as ambient conditions (noise, temperature, humidity), pH of drinking water, diet, single or group housing, and methods of animal husbandry, are critical since they can profoundly affect test outcome [13–17]. Behavioural differences have been shown to be vulnerable to housing parameters across certain inbred strains, which one group [18] demonstrated when they

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compared mice that were housed individually with group-housed mice in a behavioural test battery. In particular they found that in some strains, singly housed mice habituated to test conditions faster when the assay was measuring activity and exploratory behaviours, but showed more anxiety-like behaviours in the light–dark box and hyponeophagia tests, and less so in the elevated plus maze [18]. There is also a complex interplay of gene-environment with different forms of cage enrichment [19,20] that is apparent even with subtle modification, which was found to significantly alter behaviours across inbred strains in specific tests [21]. Previous efforts of standardisation have proven challenging and identified several parameters that are uncontrollable. A key study [15] highlighted the limitations with cross-validation between laboratories, when they demonstrated that results were relatively reproducible but, some parameters could not be absolutely replicated between the three participating centres despite equating experimental design and test apparatus.

Standardising environmental variables is necessary, but equal consideration must be given to the criteria for experimental design especially when devising behavioural batteries since sequence of testing should take into account the sensitivity of specific tests that could significantly influence the outcome of subsequent tests. One group [9] showed that some tests were more susceptible to test order than others in their test battery. Measures in the light–dark box were particularly sensitive to test order in their investigation, and acoustic startle response in C57BL/6J was also affected. Circadian rhythms can further influence performance in some behavioural tests [22–24], such as those that measure activity and anxiety. These measures may be affected by the time of day as well as the cycle-phase in which the test is carried out, particularly since mice are nocturnal mammals and therefore testing during their alternate phase could affect the natural response observed across behavioural tests.

Even when taking into account all possible confounding factors, the inherent differences that are commonly reported within and between laboratories in mouse phenotyping studies are likely to be attributable to subtle and specific inter-laboratory practices, yet in order to obtain results from which we can make meaningful inferences, adopting common standards are a prerequisite for its success. The use of clearly defined objectives and employing a robust and reliable phenotyping battery at the outset will ultimately enable results to be obtained with a reasonable level of accuracy. A demonstrable behavioural battery [25] that was developed and validated across five test centres throughout Europe, showed the value of well defined Standard Operating Procedures (SOPs) within their behavioural battery, which allowed them to identify the potential sources of variation, and where necessary refine procedures to attain a good degree of reliable and reproducible strain ranking effects between participating centres.

We devised a high throughput behavioural battery that aimed to index a broad and comprehensive range of behaviours such as anxiety, locomotor activity, learning and memory, in order to map underlying QTLs and to further characterise the biological pathways involved within a recombinant inbred panel (BXD) and an outbred population (Heterogeneous Stock) of mice. Central to our success in this task was evaluation of the behavioural battery design, which was performed using the C57BL/6J strain, a progenitor of both BXD and HS. The specific aims of the evaluation exercise were to assess how robust our experimental design was by means of the potential confounds frequently associated with behavioural batteries. We investigated the effects of several aspects of our experimental design: individually versus sibling (pair) housed; testing at different times during the day (am/pm); the test run through the battery versus testing in isolation; and the initial placement within the light–dark box. In addition, we assessed how these behavioural profiles differ in this battery of tests across 8 well characterised inbred strains, some of which are also progenitors of the HS mice we used in our study.

2. Methods

2.1. Animals

Male mice [C57BL/6J ($n=111$), 129S1/SvImJ ($n=11$), A/J ($n=11$), BALB/cByJ ($n=11$), C3H/HeJ ($n=10$), DBA/2J ($n=11$), FVB/NJ ($n=11$), SJL/J ($n=11$)] were generated in the Comparative Biology Unit animal facilities at the Institute of Psychiatry using original stocks [respective stock numbers: 000664, 002448, 000646, 001026, 000659, 000671, 000671, and 000686] purchased from The Jackson Laboratory (Bar Harbor, ME, USA). We tested male mice only throughout our battery to avoid the possible confounds of oestrous cycle effects [26,27]. Mice were weaned at 3 weeks of age and transferred at approximately 8 weeks of age to a separate housing facility where all mice were singly housed; except for a group ($n=10$) of C57BL/6J mice that were sibling housed (2 per cage) to investigate the effect of paired-housing on behavioural outcome in the battery of tests. All mice were allowed to habituate for 2 weeks before undergoing the battery of behavioural tests.

2.2. Housing conditions

Mice were housed in standard cages measuring $30.5\times13\times11$ cm, with food (Rat and Mouse No. 1 Maintenance Diet, Special Diet Services, Essex, UK) and water available *ad libitum*. The housing room was maintained on a reversed 12:12 light cycle with white lights on from 20:00 to 8:00 h and red light on during the dark cycle. Behavioural tests were performed during the dark cycle between 09:30 and 19:00 h; except for the two groups in which time of day was being investigated, where they were tested between 09:00 and 12:00 for the am group, and between 15:00 and 19:00 h for the pm group. Light intensity in the housing room was 400 lx (lux) during the lights-on period and less than 2 lx during the dark period [28]. Four red cluster lights (LED cluster red light No. 310-6757; RS Components Northants, UK) of approximate wavelength 705 nm provided minimal red light during the dark phase, allowing experimenters to work with the mice during their dark phase. Ambient temperature in all rooms was maintained at 21 ± 2 °C with 45% humidity level. Sawdust and nesting materials in each cage were changed once a week, but never on the day before or the day of testing to minimize the disruptive effect of cage cleaning on behaviour. All housing and experimental procedures were performed in compliance with the UK Home Office Animals Scientific Procedures Act 1986.

2.3. Experimental procedures and analysis

Table 1 illustrates the 12 test groups included to evaluate the behavioural battery designed. Behavioural tests in the battery (Fig. 1) were performed in the following order starting with those considered to be least stressful: barrier test, home-cage activity (HC), open field (OF), novel object exploration (NO), elevated plus maze (EPM), light–dark box (LD), primary screen of SHIRPA (*S*mithKline Beecham Pharmaceuticals; *H*arwell, MRC Mammalian Genetics Unit; *I*mperial College School of Medicine at St Mary's; *R*oyal London Hospital, St Bartholomew's and Royal London School of Medicine; *P*henotype *A*ssessment), puzzle box (PB), Morris water maze (MWM), and tail suspension test (TS). Tests in the non-battery tested groups were performed at the equivalent stage within the battery. Mice were tested in a pseudorandom order and were moved to the behavioural suite adjacent to the housing room immediately before testing with a minimal transfer time. Each apparatus was wiped clean with 1% Trigene® between subjects to avoid olfactory cueing influencing behaviours. Behaviours for all tests were recorded on videotapes for further detailed analysis. For albino mice, automated tracking using EthoVision software [29,30] was not possible and so a comparable method of hand coding was used to score locomotor activity for the OF and LD across these mice. Mice were returned to their home cage at the end of each test.