Phenomics: fiction or the future?

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The ease with which genetic mutations can be induced in or introduced into mammalian organisms, such as the mouse, has created a significant need for phenotypic analysis. Developments in computer technology, instrumentation and bioinformatics, as well as in numerous neuroscience disciplines, will help to meet the demands set by the molecular revolution. As a result, the field of 'phenomics' is being born. This will integrate multidisciplinary research, with the goal of understanding the complex phenotypic consequences of genetic mutations at the level of the organism. This paper focuses on one of the disciplines that show promising developments, behavioral science.

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Genetics has become a major driving force in biology. However, although the development of ever-more powerful and sophisticated recombinant DNA technologies is accelerating at an exponential rate, other disciplines are struggling to keep up with the pace. The task is particularly complex in neuroscience. How can we understand the function of genes in such a complicated organ as the brain of mammals, such as ourselves? A model organism, the mouse, has been chosen because of the high sequence homology of its genome with that of humans [1]. The recognition that phenotypic characterization of this species is crucial has led to the Mouse Phenome Project [2]. Grant funding agencies, including the National Institutes of Health (NIH, USA), have also issued requests for applications to stimulate phenotyping of genetically engineered mice (e.g. http://www.nih.gov/science/models/mouse/genomics/priority_setting_genomics.pdf). It has been argued that behavioral phenotyping, along with numerous other approaches, will be instrumental in analysis of the roles of genes in brain function [3]. Although behavioral analyses have been employed in molecular genetic research of the function of the brain, the behavioral studies have been slow and labor-intensive and, thus, often very narrowly focused. It is forecast that the demand for sophisticated and rapid behavioral phenotyping tools will broaden the perspective of behavioral analysis, both conceptually and technologically.

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Searching under the torchlight

Although it is generally appreciated that the ultimate output of the brain is behavior, and that behavioral analysis has the potential to reveal functional alterations of any circuit or any neurobiological process of the brain [4], serious practical issues limit the utility of behavioral studies. Most importantly, behavioral tests are time consuming and, thus, investigators often prefer to focus on particular aspects of behavior using a limited number of tests. The danger of this approach is that the ability to properly evaluate the consequences of a mutation can be significantly limited. Thus, investigators have suggested that perhaps a battery of tests should be conducted [5]. The questions of how many tests are needed and what should be included in a test battery are debated. Some think mouse behavior is relatively simple compared to that of the rat [6] and that perhaps a handful of tests would be sufficient, whereas others argue for complexity [7]. It is probable that novel mutations could induce unexpected functional changes in the brain that might not be captured by a limited number of tests. Also, consider the following. The total number of genes is limited, and so too must be the number of biological mechanisms underlying brain function: thus, ultimately, the number of behavioral traits, or 'phenes', must also be finite. However, behavior is influenced not only by the combinatorial number of genetic effects, but also by what is almost certainly an even larger number of environmental factors that interact with the genetic effects. In summary, the number of behavioral 'phenes', and the number of behavioral tests needed to quantify them properly, is likely to be enormous. Clearly, 'complete phenotyping' is only a dream.

Nevertheless, healthy compromises can be made and some behavioral test batteries have already gained recognition. The SHIRPA protocol [8] is a conglomeration of previously characterized and individually developed tests; another example is the Cambridge Neuropsychological Test Automated Battery (CANTAB) system (http://www.camcog.com) [9], which has some ingenious computerized behavioral paradigms that allow the user to compare behavioral functions across multiple species, from rat to man. The questions of how to organize and design test batteries are also now being discussed. The debate centers primarily on the issue of whether one needs standard or custom-designed tests [10–12]. One argument for standardization is clearly the ability to compare results from laboratory to laboratory [10]. However, the advantages of cross-laboratory comparability have been pitted against the issue of the rigidity of standardization, leading to an inability to evaluate properly potentially unique genetic effects [12]. The emerging consensus appears to be a compromise: one needs to have a set of standard tests, a sort-of reference point, but the need for creative thinking and custom-made behavioral applications is also recognized [11]. Numerous other questions have also been considered. For example, investigations have begun to analyze how the order of tests in a battery might influence the outcome of the behavioral study, and whether one test could interfere with another [13]. The problems associated with laboratory-specific environmental factors and their

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interaction with the genotype of the studied subjects has also been brought to attention [14], and false-positive and false-negative findings due to inappropriate control of the environment [15] and/or genetic background [16] have been pointed out. Finally, the importance of better understanding of the ecology of the mouse and utilization of biologically and ethologically relevant behavioral paradigms has been discussed [3,7,17–19] – an argument that has led to conceptually novel test paradigms (e.g. Refs [20,21]). Although some of the above questions remain controversial, test batteries offer a reasonable solution to the problem of how to characterize a phenotype.

Often, the rationale behind the test batteries is that they are organized hierarchically: the investigator starts from broader, less-specific tests that are sensitive to numerous alterations, and subsequently employs increasingly specialized tests that finally tease out the details of the functional alterations of the brain. However, the question of what to have as the organizing principle has not been addressed explicitly. Pharmaceutical and biotechnology research companies might benefit from test batteries that are primarily organized according to disease target. For example, a test battery aimed at researching Alzheimer's disease might need to cover major domains of cognition (e.g. attention, short- and long-term memory and executive function) in a manner similar to that used to examine these behavioral phenomena in the human clinic. Other research could benefit from organization of the test battery in a way that would allow one to tap into different neurobiological mechanisms. For example, in the analysis of memory, one might be interested in whether a mutation affects mechanisms of acquisition, consolidation, retention or recall. One could also be interested in which brain area is affected – for example, whether procedural learning (perhaps function of the cerebellum), relational learning (perhaps the hippocampus), elemental learning (perhaps the cortex) or emotional learning (perhaps the amygdala) is altered. Although test batteries based upon these different organizing principles are clearly not orthogonal or mutually exclusive, employing them could yield different answers. However, irrespective of the organization of the test battery, it has been generally appreciated that multiple tests tapping into the same behavioral trait, but employing idiosyncratic performance demands, must be conducted to avoid false findings [3].

Fig. 1. The SmartCube™ system (PsychoGenics, Inc., patent pending) will be the first to automatically and systematically capture, quantify and store information on a large number of behavioral motor and posture patterns exhibited by genetically altered or pharmacologically manipulated mice in a high-throughput manner. The SmartCube™ will incorporate robot-like hardware, computer vision, neurological assessment tools and machine-learning algorithms to capture and integrate behavioral and physiological parameters. In addition to the usual measures of frequency, duration and intensity of numerous behaviors, the system will also analyze complex patterns such as transition matrices (i.e. the temporal succession of different behaviors). Physiological parameters, such as heart rate, can also be quantified, in parallel with the acquisition of behavioral data. (With permission from PsychoGenics Inc.)

Speed versus quality

From the above it is clear that a test battery represents a significant challenge. Optimization of a battery is not trivial. The primary problem is a practical one: behavioral analysis is space- and time-intensive. How can one collect all the pieces of information with which to properly evaluate which aspects of brain function are affected by a genetic manipulation within a reasonable amount of time? Would speed be increased at the expense of quality? Steps have been taken to address this problem. One solution is scalability (i.e. the increase of the number of pieces of apparatus that can run in parallel). The second is to increase the information density of the test (i.e. to increase the number of behavioral measures of brain function one can obtain from a single test). The third solution is to increase the flexibility of the test apparatus, so that it can tap into a broader spectrum of brain functions. Increased processing speed and memory capacity now allow computers to control several pieces of apparatus in which animal behavior is monitored and also make it possible to record numerous variables at once. For example, one commercially available system using force-transducer technology monitors eight chambers at a time and can be programmed to record a large number of behavioral variables (MED Associates, Vermont, USA). The system has been modified by Fitch *et al.* [22] to quantify movements of the mouse. Methods of extracting information from particular force-prints (changes in the waveforms of acceleration forces that the mouse generates while moving) are being developed, with the aim of identifying and measuring numerous motor and postural patterns – the elements of the ethogram. The chambers can be equipped with a range of

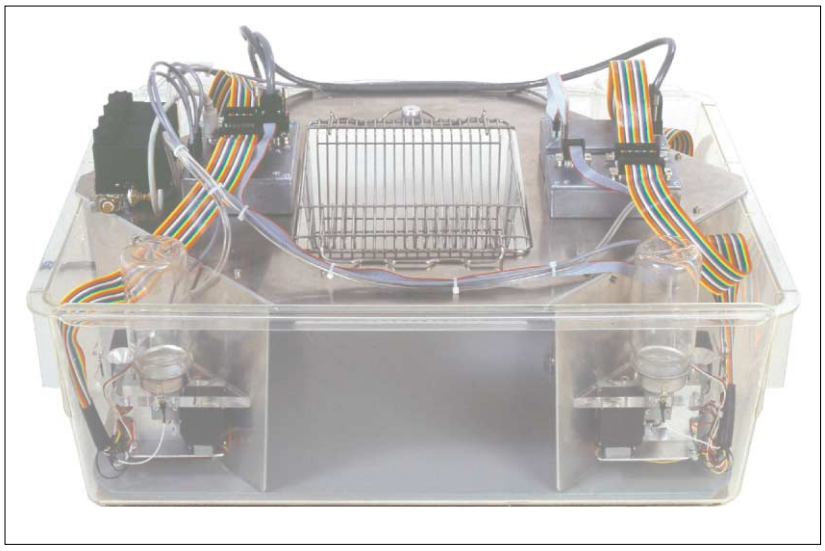

Fig. 2. The IntelliCage (NewBehavior Inc.) allows a fully automated and multidimensional longitudinal assessment of mnemonic characteristics of mice in a group-housing setting. Up to 16 transponder-tagged mice can be housed and monitored in the IntelliCage. The apparatus contains four learning corners that include antennae decoding implanted microchips, infrared detectors and photocells to monitor the whereabouts of each mouse. The corners also have two openings for nose-pokes, two servo-operated gates either blocking or permitting access to liquid rewards, light diodes above the openings, two integrated 'lickometers', an opening to deliver air-puff punishment and a temperature sensor. Inputs from sensors and outputs to motors and air-valves of the IntelliCage are handled by microprocessors that communicate with a central computer linked to the remote user by intra- or internet. The central computer stores events and controls delivery of stimuli according to pre-programmed task parameters and/or the learning histories of the subjects. Application modules include, for example, spatial preference or avoidance, visual discrimination learning, taste preference or aversion, various operant conditioning schedules and spatiotemporal conditioning. Progressive age-dependent deterioration of memory can also be monitored longitudinally, and social interactions, such as competition for access to liquid, can be assessed. (With permission from NewBehavior Inc.)

accessories allowing, for example, analysis of different forms and stages of memory and fear. Even more sophisticated are techniques based upon video-image analysis. These systems [which are under development in, for example, the laboratories of PsychoGenics Inc. (Terrytown, NY, USA) and are similar to approaches pioneered by Noldus Info. Tech. B.V. (Wageningen, The Netherlands)] [23,24] will allow one to identify, interpret and quantify 3D images of the freely moving mouse. Thus the classical premise of ethology (i.e. that seemingly continuous behavior can be broken down into discrete behavioral elements) will be put to good use: proper quantification of behavioral elements will be possible without having to watch videotapes and score behavior manually. The behavioral apparatus in which the computer based image analysis is done can also be made fairly complex. The SmartCube™ (Fig. 1) can be equipped with numerous instruments: for example, food receptacles, shock grids and conditioned-stimulus delivery devices can all be attached. Because behavioral quantification, as well as stimulus presentation, is done by the computer, one can design pavlovian or operant conditioning paradigms in a precisely controlled manner and without the interference of a human experimenter (who is often regarded by the experimental subjects as a dangerous predator) [4]. Other devices, based on the principles of scalability, increased information density and flexibility are also being developed. IntelliCage (Fig. 2), the first prototypes of which have already been made commercially available by NewBehavior Inc. (Zurich, Switzerland, http://www.newbehavior.com), employs transponder-based technology to monitor the whereabouts of several mice in the same (intelli)cage. As the device can differentiate individual mice by means of implanted, commercially available microchips, the computer-based monitoring system can tell which mouse is working to obtain reward from the receptacle, which one is running in the middle of the cage and which one is sitting in the corner frustrated by all this technological sophistication. Although it might not be apparent at first glance, the IntelliCage is not only sophisticated but also mimics the natural habitat, a mouse community. In fact, it has been developed on the basis of information gathered in field studies [25]. This semi-natural enriched environment could, thus, facilitate the high-throughput analysis of several behavioral phenomena, ranging from learning or anxiety to numerous aspects of social interaction.

Bioinformatics to the rescue

The amount of data one gathers using such devices can be staggering. Bioinformatics tools, multivariate statistical methods and pattern analysis can be required to extract information from these complex behavioral experiments properly and concisely. Furthermore, phenotyping is not the exclusive domain of behavioral science. For example, *in vivo* multi-electrode recordings from individual neurons have already shown great promise [26]. The fact that behavioral and electrophysiological quantification of brain function can be conducted *in viv*o in the same, freely moving mouse will further increase the demand for complex mathematical procedures in data analysis and data mining [27]. It is possible that patterns based on behavioral 'mosaic pictures' will emerge and that these patterns might reflect certain neurobiological mechanisms or disease states better than the individual measures that are traditionally quantified. Clearly, investigation of complex behavioral phenomena will require new analytical procedures.

Comprehensive databases will help the investigator. The seeds of such databases already exist. For example, the internet-accessible public domain Induced Mutant Resource (IMR) database (http://www.jax.org/resources/documents/imr/) and Transgenic/Targeted Mutation Database (TBASE) (http://tbase.jax.org/) from The Jackson Laboratory (Bar Harbor, ME, USA), and the Mouse Knockout and Mutation Database from BioMedNet (http://research.bmn.com/mkmd), provide comprehensive listing of mutant mice along with their phenotypic profiles [28]. Open discussion forums, where scientists can share data and ideas beyond the details usually provided in

peer-reviewed journals, have also been suggested in the literature [29] and at a seminal conference, 'Behavioral Phenotyping of Mouse Mutants 2000', held in Cologne, Germany. Bioinformatics tools that were developed to cope with the large amount of genetic information coming out from sequencing of genomes or from gene expression analyses [30] will be also crucial for organizing and interpreting phenotypic data.

The term 'phenomics' is coined to describe, in anticipation, the new field that is likely to form from the behavioral and other phenotypic analyses designed to obtain a large amount of information on the varying effects of genetic mutations. This field will be defined by not only sophistication of the experimental paradigms but also technological complexity. Hardware and software engineers, as well as behavioral (and other) neuroscientists will co-develop test paradigms and equipment that will enable investigators to cope with the demands set by the increasing number of mutants generated by such techniques as transgenics or chemical mutagenesis. Phenomics will be a crucial approach in academic, as well as industrial, research and could lead to a significant paradigm shift both in the genetic analysis of brain function and in drug development.

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