

Neurobehavioral assessment in the information age

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The elucidation of the human and mouse genomes provides new opportunities for exploring the genetic underpinnings of complex mammalian behaviors. This information also provides new windows into the pathophysiology and treatment of neuropsychiatric diseases. Optimal use of the rapidly escalating numbers of mouse lines engineered for these purposes is hindered, however, by practical and theoretical limitations of common behavioral analyses. New strategies combining automated behavioral monitoring and information technologies are currently under development in both academic and industrial settings. These hold promise, both for improving the throughput of mouse behavioral assessment and for providing new insights into the neurobiology of mammalian behavioral regulation.

Understanding the neural mechanisms of complex behavior, as well as the abnormalities in behavior that accompany neuropsychiatric disorders, represents one of the most formidable challenges in biomedical research today. Our very limited knowledge of the neural basis of behavior means that this endeavor must rely, to a unique extent, on *in vivo* studies. The human and mouse genome projects, coupled with an impressive array of molecular genetic technologies to manipulate expression of individual genes within the brain, have provided unprecedented opportunities for investigating the influence of genes on behavior. In contrast, behavioral assays, and their application to large numbers of animals, have not kept pace in terms of throughput with the rapidly escalating use of genetic manipulations in mice. There is also a need for more advanced behavioral assays to screen increasing numbers of potential medications targeted to a large array of neural proteins. Here we briefly describe this challenge and review some of the innovative solutions that are now in development to relieve the bottleneck in behavioral assessment. Such approaches may not only improve throughput, but also provide new insights into the regulation of rodent behavior that are not approachable with conventional assays.

Types of behavioral assessments

Behavioral assays in psychopharmacology have been categorized into three classes¹: animal models of clinical disorders, behavioral screening

tests and behavioral bioassays. Animal models of clinical disorders represent attempts to simulate symptom clusters characteristic of particular diseases. Screening tests are used to assess the impact of genetic and pharmacological manipulations on behaviors chosen to reflect particular behavioral processes of interest. For example, the forced swim test and elevated plus maze are used to screen compounds and genetic mutations for their relevance to depression² or anxiety^{3,4}, respectively. Alternatively, some behaviors are used as bioassays to assess the activity of particular neural pathways. Examples of such behavioral bioassays include circling behavior to test function of the brain's dopaminergic systems, and head-twitch responses to serotonin receptor agonists as a measure of serotonin 5-HT₂ receptor sensitivity^{5,6}.

Multiple behavioral assays have been used for decades to learn a great deal about the neural basis of behavior. It has become apparent, however, that there are fundamental limitations in their application to neural processes related to neuropsychiatric disorders and CNS drug discovery^{7,8}. Although *bona fide* animal models of neurologic diseases are becoming increasingly available as known disease-causing genetic factors are reproduced in mice, such models are not available for most psychiatric conditions because of the relative lack of knowledge of the relevant etiological factors (for example, see ref. 9). In addition, it has become increasingly clear that accurate interpretation of individual behavioral tests can only be achieved in the context of a thorough set of behavioral assessments, which are not used in the vast majority of publications in the field.

Animal models of psychological processes

Genetic and pharmacological influences on psychological processes must be inferred from their effects on observable behavior, but several factors complicate our ability to draw inferences about psychological state from behavioral data (Box 1). The elevated plus maze and Morris water maze are good examples of this. The plus maze, a commonly used test of anxiety-related behavior, measures a rodent's avoidance of open or brightly lit places. In this test, animals are given a choice between narrow elevated platforms that are either walled or open³. The predisposition of animals to prefer the walled arms is thought to correlate with anxiety state. The assay has some predictive validity for the identification of compounds with anxiolytic actions in humans. Like many rodent behavioral assays, the plus maze was originally validated in rats, and more recently adapted to mice.

This is also the case for the water maze, a commonly used assay of spatial learning and memory^{10–13}. In a typical procedure, animals are placed within a circular pool of opaque water—to escape, they must swim to a submerged platform. If the platform position remains constant, the animal's performance improves over the course of repeated trials, as indicated by reduced latencies to find the platform. Presumably, efficient performance in this task reflects a spatial memory

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BOX 1 EXPERIMENTAL APPROACHES TO BEHAVIORAL ASSESSMENT

Approach	Advantages	Disadvantages
Standard tests	<ul style="list-style-type: none"> • Extensive pharmacological validation available • Relatively simple and rapid to administer 	<ul style="list-style-type: none"> • Risk of data misinterpretation • Limited construct and face validity • Certain assays have low throughput • Serial tests have very low-throughput and require large numbers of animals
Batteries of standard tests	<ul style="list-style-type: none"> • Pharmacological validation available • Reduced numbers of animals required • Data interpretation improved by assessing diverse behaviors 	<ul style="list-style-type: none"> • Limited construct and face validity • Certain assays have low throughput • Test order may confound results
Naturalistic assays	<ul style="list-style-type: none"> • Behaviors assessed in a more ethologically valid context • Reduces misinterpretations of behavioral responses • Wide range of complex species-characteristic behaviors may be elicited 	<ul style="list-style-type: none"> • Low-throughput • May be time-consuming to establish assay conditions for certain behavioral processes • Lack of widespread experience with these approaches for mice • Requires extensive pharmacological validation
High-throughput screens	<ul style="list-style-type: none"> • Capable of very high throughput • Potential for detecting novel behaviors 	<ul style="list-style-type: none"> • Limited construct or face validity • Some rely on limited tests, increasing incidence of false positives and false negatives • Requires extensive pharmacological validation
Quantitative home cage behavior analysis	<ul style="list-style-type: none"> • Effects of handling and novelty minimized • Assesses multiple behaviors over long time intervals • Potential for detecting novel behaviors • Relatively low labor costs 	<ul style="list-style-type: none"> • Need for chronic housing links throughput to number of available cages • Requires substantial computational resources • May be time-consuming to establish assay conditions for certain behavioral processes • Requires extensive pharmacological validation

process engaging a navigation strategy guided by visual cues. Spatial learning is verified in a subsequent 'probe trial', where the animal swims during a trial with the platform removed; preference for the prior platform location is viewed as a measure of spatial learning. Drugs that disrupt cognitive function impair performance in the water maze.

There are several sound reasons for the popularity of the elevated plus maze and Morris water maze. Compared with other assays of similar psychological processes, these tests are simple and rapid to perform. The tests also have been pharmacologically validated, as mentioned above. Moreover, a large literature addresses the contributions of neural circuits that regulate an animal's performance in these assays.

Nevertheless, there are a number of problems with the manner in which such tests are sometimes performed and interpreted. One limitation relates to a lack of accepted and standardized protocols. Inter-laboratory variations often exist in both the testing apparatus and procedures used. Too often, results from one group cannot be compared to the results from another group because of such methodological differences^{8,14}. A second limitation is that the results of individual behavioral assays may be readily misinterpreted when they are conducted in isolation. For example, an undetected cognitive deficit interfering with the

processing of contextual cues could cause abnormal performance in the elevated plus maze. Potential perturbations of sensory perception (e.g., blindness), activity levels, or motor coordination could also lead to misleading results. Analogously, performance in the water maze can be influenced by many factors, including prior testing experience, motor impairments, abnormal stress responses, visual problems and perturbed thermoregulation^{13,15}. These considerations underscore the importance of not relying on any one assay in making a claim regarding the psychological basis of a behavioral phenotype. The evaluation of a diverse array of behavioral domains improves the extent to which each individual assay may be correctly interpreted.

One common approach to obtaining a comprehensive behavioral assessment is to run cohorts of mice through batteries of multiple behavioral assays (Box 1). Examples include the SHIRPA protocol¹⁶ and PsychoScreen (PsychoGenics, Inc.; www.psychogenics.com/services/psychoscreen.htm), which involve an initial neurologic assessment followed by a series of standard tests of motor function, analgesia, stress responses and cognition. Although this is a practical approach, particularly given the limited numbers of mutant mice or amount of novel compounds typically available, behavioral test batter-

ies present an additional set of caveats. First, they are time consuming and labor intensive, which limits their throughput, as will be discussed below. In addition, the extent to which prior behavioral testing influences test performance is often not examined. Recent studies, for example, have compared the performance of naive mice in the Morris water maze and elevated plus maze with the performance of animals in which these tests were administered within the context of a behavioral battery. In one case, mice tested in the water maze within a battery did not show spatial selectivity for the platform location, whereas naive mice did¹⁵. In the elevated plus maze, C57BL/6J mice displayed large differences in open platform time depending on whether they were tested within a battery or in the naive state¹⁷. In the latter study, the test battery did not alter elevated plus maze behavior in 129S2/Sv mice, indicating that testing order can interact with genetic background to further complicate the interpretation of behavioral phenotype data. Thus, limitations of individual assays and behavioral batteries of tests highlight the need for innovation.

Relevance of an ethological perspective

One way to improve the interpretation of behavioral tests is to consider them within an ethological perspective. As developed in the mid-twentieth century by investigators such as Konrad Lorenz, Niko Tinbergen and Karl von Frisch, the discipline of ethology focuses on detailed analyses of the diverse sets of behaviors seen in animals in their natural habitats. Connotations of the term “ethological” vary widely among investigators, and some would find it inappropriate to apply the term to studies in laboratory settings with genetically defined animals that do not exist in the wild. Regardless of whether the term may be applied to such studies, their interpretation may nevertheless be enhanced by insights that have been provided by ethologists. Often, standard behavioral assays are considered in a rigid manner without regard to species-typical behaviors seen during testing. For example, mice placed directly on the open arm of the elevated plus maze may show a freezing response, recognized by ethologists as a characteristic response of rodents to threatening stimuli¹⁸. Although this could result in increased open arm time, it would be a mistake in this case to conclude that the animal showed reduced anxiety-like behavior. Ethological considerations can also complicate attempts to adapt rat behavioral assays for mice. As just one example, differences in the natural habitats of rats and mice have been implicated in the relatively poor performance of mice in the Morris water maze procedure. Given that rats may be better adapted to wet habitats, their superior performance in this task may relate more to task suitability than to cognitive differences^{19,20}.

Several groups are now applying naturalistic approaches to the analysis of mouse behavior¹⁸. For example, a population of mice was derived from the offspring of several laboratory strains that had been released into a 400 m² outdoor pen in Western Russia, and subjected to natural selection²¹. Experimental subjects were captured in the enclosure, implanted with microchips for tracking, and re-released into the pen, which contained automated feeding monitors. The system enabled observations relevant to foraging strategies, social organization and diurnal patterns of activity. More recently, a smaller modified version of this approach was used to reveal phenotypic perturbations of the flexibility of foraging strategies in *trkB* receptor mutant mice²². (*TrkB* is a neurotrophin receptor implicated in cognitive function and mood²³.) Such approaches provide opportunities for insights into mouse behavior that are not available under standard laboratory conditions.

Another example is the Mouse Defense Test Battery, used for assessment of anxiety-related behavior²⁴. In this assay, mice are placed in a test alley and approached with an anesthetized rat, a natural predator of mice that triggers unconditioned aversive reactions. A series of species-

typical behaviors is assessed, including flight, freezing, risk assessment (for example, repeated episodes of extending and withdrawing body in the direction of threat), and defensive threat and attack responses. Factor analysis of these behaviors showed four factors that were differentially modified by a series of anxiogenic and anxiolytic drugs. Such approaches that more fully account for the variety of behaviors seen in mice in threatening situations hold promise for modeling several aspects of anxiety responses. However, practical challenges to implementing such naturalistic approaches for high-throughput screening have limited their widespread use¹⁸.

Mouse models of neuropsychiatric disorders

A major determinant of the quality of an animal models of neuropsychiatric disease relates to the degree to which it reproduces the pathophysiology of the corresponding human disorder—that is, its ‘construct validity’. Mouse models have been particularly useful for studies of disorders whose underlying genetic causes have been identified in humans (e.g., neurodegenerative diseases such as Alzheimer, Parkinson and Huntington diseases, amyotrophic lateral sclerosis, hereditary ataxias, frontotemporal dementias, multiple sclerosis and prion diseases^{25–27}). Mice with disease-causing mutations show some, but not all, of the neuropathological and behavioral abnormalities seen in humans, and these abnormalities have served as critical endpoints for assessing the potential clinical utility of novel therapeutic agents^{28,29}. These mutant mice have also been useful in genetically dissecting individual steps in the biochemical pathways responsible for disease pathogenesis. Most behavioral analyses of these models have focused on assays of motor function and cognition. It is noteworthy that many of these disorders also involve abnormalities in affect, diurnal rhythms and impulsivity that may precede cognitive and motor symptoms. Accordingly, a recent study of mice with scrapie and bovine spongiform encephalopathy revealed impairments in circadian activity and open-field exploration prior to the onset of motor abnormalities³⁰. It is therefore likely that the application of comprehensive behavioral analyses to these models will improve their sensitivity and promote insights into the pathophysiology and treatment of these neurodegenerative disorders.

In striking contrast, the prospects of developing *bona fide* animal models for most psychiatric diseases are complicated by at least four major considerations^{31,32}. First, in comparison to neurodegenerative diseases, there is a relative paucity of definitive information regarding causal genetic factors, making the generation of mouse models difficult. Second, the precise contribution of environmental factors to the pathophysiology of these disorders remains poorly understood, precluding the production of convincing models through environmental manipulation. A third consideration relates to clinical diagnostic limitations—a great deal of heterogeneity exists among the symptom clusters of individuals sharing a common diagnosis (such as depression or schizophrenia), and many are not readily classifiable using standard criteria. Uncertainty regarding the clinical features of the human syndromes to be simulated obviously complicates the validation of animal models. Finally, many of the hallmark features of the human disorders pose a daunting challenge for the mouse psychiatrist. Unanticipated breakthroughs would be required to convincingly model phenomena such as guilt, religiosity, grandiosity, envy, delusions, hallucinations, grief, body image distortion, and multiple personality in the mouse.

One partial exception to these limitations is drug addiction, where at least one of the key etiological agents—the drug—is clearly identifiable, and where key features of the behavioral pathology (drug self-administration) is readily measured in rodents^{33–35}. Progress has also been

made in modeling cardinal symptoms of human addiction, such as drug craving, in rodents^{36,37}, although assessing whether a mouse 'wants' a drug remains challenging.

Although most behavioral models of psychiatric disorders, other than addiction, are unconvincing as disease simulations, they may nevertheless prove useful for exploring the neural basis of particular manifestations of these disorders^{31,32,38}. Thus it is possible to model specific symptoms of a psychiatric illness (loss of appetite, disrupted sleep) even if modeling the entire syndrome is not yet feasible. In addition, many available behavioral tests have predictive value for potential medications. This can be illustrated by the forced swim test, an assay used to screen antidepressant compounds². Animals are placed in a water-containing cylinder from which they cannot escape. Initially, mice make apparent attempts at escape, but over time these give way to periods of immobility when they float at the water surface. This immobile state is proposed to reflect 'behavioral despair'—the loss of hope of escaping. Compounds that reduce immobility times have an increased likelihood of antidepressant efficacy in humans. Nevertheless, the face and construct validity of this test—that is, the extent to which the mouse resembles a depressed individual, or the centrality of swim stress to human depression—is unconvincing, particularly because immobility could be viewed as an adaptive strategy in this experimental context. Therefore, if a mutation is found to result in decreased immobility time, it is risky to conclude that the mouse is 'less depressed'. A more conservative interpretation would be warranted—that the animal has a behavioral abnormality associated with responsiveness to swim stress and antidepressants. Despite these limitations, the forced swim test has some predictive validity, indicating that an understanding of its mechanistic basis may shed light on the function of neural pathways pertinent to the treatment of depression.

Behavioral screening: the problem of throughput

The need for high-throughput behavioral screening procedures is illustrated by two endeavors: (i) the application of large-scale mutagenesis approaches for detecting genes that regulate behavior, and (ii) the use of behavioral testing for CNS drug discovery. Enthusiasm for chemical mutagenesis approaches has led to the establishment of international mutagenesis centers that include behavioral screening within their panels of phenotypic assays^{39,40}. The thousands of mutants produced yearly by these programs could provide an invaluable resource for the exploration of genetic influences on behavior. Currently, limitations in the availability of comprehensive behavioral test strategies that may be administered in a high-throughput manner creates a substantial bottleneck in our ability to use the valuable animal resources provided by these centers.

CNS drug discovery differs most from other therapeutic areas by its unique reliance on *in vivo* tests for target identification and validation and for compound selection and refinement. As stated earlier, a comprehensive behavioral assessment, which is needed for accurate interpretation of behavior, is a very slow, costly, and sometimes subjective process. Consequently, at a time when the number of potential targets and the number of compounds directed toward these targets are exploding, behavioral screening has become the main bottleneck in CNS drug discovery⁴¹.

Recently, we have seen significant improvements in the throughput of behavioral screening (Box 1). Several companies have developed semi-automated systems for measuring multiple behaviors, such as locomotor activity, fear/anxiety responses and startle. The currently available devices, however, are not integrated into databases designed to coordinate the analysis of multiple behavioral responses in order to predict clinical efficacy. Some private companies are moving in that

direction. For example, Hypnion is developing a high-throughput system that monitors electroencephalograms, sleep, and several physiological variables in rodents (www.hypnion.com/discovery/score.htm). Their SCORE-2000 system uses a large database to predict efficacy and side-effects of existing and experimental hypnotic agents. SmartCube (PsychoGenics), one of the most comprehensive approaches to date, works by compressing numerous tests into one by gathering comprehensive, detailed, and fully-automated data into a large database, and by implementing sophisticated data-mining algorithms to detect psychotropic drug activity⁴¹. Mice are given a drug and placed in SmartCube for a designated time, during which they are presented with a sequence of stimuli. Videotracking and other automated measures are then processed by SmartBase, an intelligent database that, through sophisticated statistical analysis and data-mining, identifies 'drug-signatures'—for example, antidepressant, antipsychotic or anxiolytic effects. Further research is needed to determine whether it will be possible to generate such drug signatures by a high-throughput method.

High-throughput behavioral tests could markedly increase the speed of compound screening, and reduce the subjectivity inherent in many current behavioral tests. However, a key drawback must be overcome. The automated tests will probably pick up novel features of an animal's behavior that do not have the extensive predictive validity of standard behavioral assays. The elevated plus maze, Morris water maze, and forced swim test, for example, may be labor intensive, but they are time proven. Extensive pharmacological validation will likely be needed to convince a pharmaceutical company to pursue such a novel line of drug discovery. Likewise, to understand how a drug or mutation affects behavior, any finding from a high-throughput assay would require much more investigation to elucidate the underlying behavioral and neurobiological mechanisms involved.

Quantitative home-cage behavioral pattern analysis

In addition to the high-throughput approaches to drug discovery described in the preceding section, efforts are also underway to apply information technologies for a comprehensive evaluation of the impact of experimental manipulations (mutations, environmental manipulations, drugs) on mouse behavior. At the University of California, San Francisco, an initiative is underway for the collection and mining of data, reflecting the diverse and spontaneous behaviors of mice in their home cages (Box 1)⁴². Animals are fully acclimated to housing in cages containing nest enclosures, food-intake monitors, lickometers and activity platforms that record animal position and movement velocity with high temporal and spatial resolution. Temporal and spatial organization of multiple behaviors are simultaneously assessed using a variety of computational approaches, revealing substantial heterogeneity in the manner in which behavioral patterns are influenced by single-gene mutations, drugs and environmental manipulations. In association with the Jackson Laboratory Mouse Phenome Project Initiative⁴³, we have also observed that behavioral patterns vary markedly among the 16 genetically diverse inbred strains that have been examined to date (Tecott, L.H. *et al.*, *Appetite Abstr.* 40, 363, 2003). Such information will be useful as a basis to search for genes conferring strain differences and for the selection of optimal strains for the study of particular behavioral processes.

Several advantages may be associated with the use of quantitative home-cage behavioral analysis. First, it helps eliminate the confounding effects of environmental novelty, as well as the impact of handling on stress and experimental variability. It also enables investigators to monitor processes regulated over longer time scales, such as circadian rhythms, patterns of ingestion, estrus cycle influences and gradual-onset pharmacological and genetic effects. In addition, it provides an oppor-

tunity to monitor the impact of experimental manipulations in the context of the integrated expression of multiple behavioral domains. Furthermore, hypothesis-independent data mining may reveal novel insights into the regulation of behavioral patterns. Once these behavioral patterns are established, environmental modifications may be introduced to explore additional domains of behavior (such as introduction of novel objects, predator stimuli, running wheels, and so on).

On the other hand, several challenges may complicate the use of such approaches. For example, the establishment of stable behavioral patterns and the collection of sufficient data for modeling may require several weeks of monitoring. Large studies requiring analysis of terabytes of generated data require a substantial investment in computational resources. Furthermore, accurate interpretation of the functional relevance of some behavioral effects may initially require validation using standard assays.

Conclusions

The examples provided above reflect a growing appreciation within academic and industrial settings of the intricacies and importance of behavioral analysis. This is driving a proliferation of data acquisition and data-mining innovations that will bring comprehensive mouse neurobehavioral assessment into the Information Age and markedly enhance the value of mouse models for the study of neuropsychiatric disorders. Future research is needed to increase the throughput and consistency of such comprehensive behavioral analyses, as well as to incorporate ethological perspectives for an increasingly accurate view of mouse behavior. Such advances will greatly enhance efforts to relate genes to complex behavior and to optimally mine the genomic revolution for improved treatments of neuropsychiatric disease.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Neuroscience* website for details).

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