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SHORT REPORT

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Hierarchical strategy for phenotypic analysis in mice

Introduction

Neuropsychiatric disorders are a major health concern. Diseases such as Alzheimer's disease or HIV-associated cognitive and motor disorder, as well as feeding disorders, depression and the effects of drug and alcohol abuse, are far-reaching. It is becoming increasingly apparent that these and many other neuropsychiatric disorders result from the contribution of genetic or environmental factors, or a combination of the two. Unraveling the complexities of the molecular interactions in the brain and determining the precise roles of specific host genes to the development of brain dysfunction represents a major research objective. The genetically manipulated mouse has become a valuable tool in these research efforts.

In the realm of behavioral science, mutant mice have begun to increase our knowledge of the genetic, molecular and cellular mechanisms in the brain that are linked to a variety of mental disorders as well as normal behavioral processes such as stress responses, energy balance and learning and memory (e.g., Contarino et al. 1999). Moreover, the potential for current technology to develop more precise animal models of specific human neuropsychiatric disorders will likely lead to the identification of novel targets for therapeutic intervention and facilitate the preclinical testing of new therapeutic agents. Advantages of genetic methods include the ability to manipulate systems for which selective pharmacological ligands do not exist. A second strength is the behavioral sophistication by which complex processes involved in the psychopathologies associated with central nervous system dysfunction can be studied in paradigms in laboratory animals that model the human situation.

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Hierarchical strategies for phenotypic analysis can be used for multi-level analysis of behavior in mice (Rogers et al. 1997; Crawley 1999). These schemes consist of broadly based domains and tasks in order to be able to detect wide-ranging phenotypic changes. Mouse behavioral repertoires have been well characterized and thus they represent excellent subjects for phenotypic characterization of the nervous system and behavior. Like other species of laboratory animals, mice can be trained in a variety of behavioral paradigms. A powerful method for perturbing behavior and exaggerating potential differences between experimental groups involves the use of pharmacological agents (Gold 1996). Secondary screening can employ drugs possessing known neurobehavioral actions to probe specific neurotransmitter systems. Such approaches are sensitive for screening mice with targeted mutations for diverse genes and functions as well as for detecting the extensive array of phenotypes expected in mice created through random mutagenesis techniques.

Classic behavioral domains, appropriate tasks for assessment and rationale for their application are described below according to one evaluation scheme outlined in Fig. 1.

Observational phenotypic assessment

Phenotypic analysis in adult or neonatal mice provides a systematic method for comprehensively assessing and quantifying behavioral and physiologic status. Standard neurobehavioral protocols involve brief screening procedures reminiscent of a general neurological examination in human patients. They include monitoring of simple reflexes (inhibition and emergence) and each test provides information about the pattern of function of a particular system (Rogers et al. 1997; Crawley 1999). Observational screening techniques permit an initial assessment of gross gain or loss of function and these primary observations can point to further exploration of increasingly complex behaviors.

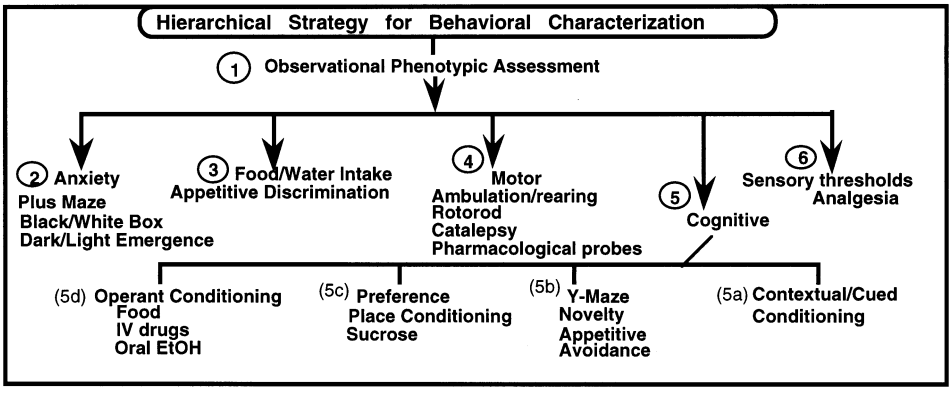

Fig. 1 Flow chart for mouse phenotopic analysis. Behavioral output requires the coordinated action of complex processes and thus a hierarchical strategy consisting of broadly-based domains is employed. Evaluation of behavioral profiles across domains aids in interpretation of results. Within behavioral domains several different experimental approaches are examined to be able to assess task-specific results versus convergence across multiple paradigms

Anxiety

Basal reactivity to stressors and novelty/exploration can be characterized in the plus-maze, black/white box and light/dark emergence paradigms. The plus-maze and black/white box have been used to reliably quantify the level of fearfulness or reactivity to a stressful environment in both rats and mice (Crawley 1999). In all of these paradigms, the exploratory drive of rodents is directly challenged by the fear of open, lighted places. Depending on test conditions either the aversion associated with open places or due to the bright light conditions can be emphasized. These paradigms do not require training but rather are based on spontaneous, unconditioned behaviors emitted by mice. Factor analysis suggests that different experimental paradigms measure distinct aspects of "emotionality" which load onto independent factors (Belzung and Le Pape 1994).

Food/water intake

Monitoring of basic physiologic parameters are important aspects of any screening program. Food intake and body weight gain are critical aspects related to the general health of the animals. Differences in food consumption can also importantly interact with performance in food motivated tasks. In general, mice are weighed before each behavioral manipulation and at the very least a minimum of once per week during experimentation. These data will provide important information on body weight gain over time and are useful for generating normal body weight curves across strains and sexes for comparison with mutant animals, as well as animals that are episodically included in studies involving food restriction. Food and water consumption can be simply monitored using a home cage assessment of intake. More sophisticated measurement of circadian topography is also possible using automated systems whereby additional information on the pattern of intake is obtained permitting more precise determination of regulation and disruption in circadian rhythmicity.

Motor activity

Several procedures can be used to evaluate the function of neural systems mediating motor output. Spontaneous motor activity is thought to reflect exploratory drive, reactivity to novelty and general level of arousal. Alterations in motor activity may indicate changes in these constructs that may then influence other behaviors. Moreover, many drugs that have positive reinforcing effects in humans produce locomotor stimulation in mice. Rotorod balancing requires a variety of proprioceptive, vestibular, and fine-tuned motor abilities. The mouse must balance on a rotating rod, which gradually accelerates, during a short test (Carter et al. 1999). This task detects drug-induced changes in motor coordination, as well as, drug neurotoxicity and developmental abnormalities and can be used to screen for motor deficits that may influence performance in other behavioral tests. Cerebellar dysfunction, vestibular problems and general muscle weakness would be manifest on this task. Catalepsy, the absence of all body movement, is a behavior produced in many different species by a variety of drugs but it is especially well characterized in rodents treated with opiates, neuroleptics and cannabinoids. Neurological disturbances of the transmitter circuits subserving these drug effects may also result in cataleptic mice.

Cognitive

Cognition, which is the culmination of the function of several underlying processes, can be examined in behavioral tasks that require integration of motivational, sensory, learning, memory and/or motor processes. Multiple tasks of complex learning ability are employed, in order to provide a convergence of information supporting dysfunction in a specific brain site or impairment in a functional construct (Wehner et al. 1996). Both spatial (Y-maze tasks; Morris water maze) and non-spatial learning (operant responding, contextual/cued conditioning) can be evaluated in tasks which measure both classical and instrumental learning processes. Fear conditioning is a form of associative conditioning where animals learn to "fear" a previously neutral stimulus simply because of

its temporal association with an aversive stimulus, such as a foot shock. Operant conditioning is used to test acquisition of new learning, long-term memory and discrimination learning. Self-administration procedures permit assessment of the direct reinforcing properties of drugs and alcohol and provide a model of drug seeking behavior in humans. The mouse models of intravenous drug and oral ethanol self-administration allow systematic investigation of the role of individual differences in substance abuse vulnerability, particularly as regards a genetic contribution (Crabbe and Phillips 1998; Pich and Epping-Jordan 1998). Preference for rewarding stimuli can also be assessed using gustatory (sucrose preference) and place conditioning paradigms.

Sensory thresholds

These procedures can determine the influence of genetic factors in nociception (Mogil et al. 1999). Protocols that measure pain thresholds are critical for assessing the relative sensitivity of different strains of mice to normal stimulation and under drug conditions. In addition to assessing hyper and hypo-analgesia, these tests allow the examination of comparability of learning among strains. For example, differences in learning among mouse strains may be due to variations in thresholds for response and motivation in the learning tests using aversive stimuli. Evaluation of other sensory systems can help to point to functional alterations that can influence behavioral output across domains.

In summary, examination of mice using a hierarchical strategy consisting of multiple assays maximizes the information gained from each phenotyped animal and more importantly aids in interpretation of results across tests. Complex traits related to normal or abnormal nervous system function and behavior including, cognition, circadian rhythms, appetite, hedonic capacity, motor behavior and pain sensitivity can be assessed. Behavioral traits related to substance abuse can also be included for study. This comprehensive evaluation of behavioral performance includes tasks that represent both spontaneous, unconditioned behaviors, as well as conditioned, learned behaviors. Most behavioral domains are examined using several different experimental approaches because each individual protocol has its own limitations. Thus, conclusions are not drawn simply from one behavioral test, but from multiple tests included in each category. Complex behavior requires the functional integration of multiple brain regions and circuits, and convergence of evidence across tasks provides the framework for interpreting individual results and developing general conclusions.

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