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A Proposed Test Battery and Constellations of Specific Behavioral Paradigms to Investigate the Behavioral Phenotypes of Transgenic and Knockout Mice

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Behavioral phenotyping of transgenic and knockout mice requires rigorous, formal analyses. Well-characterized paradigms can be chosen from the established behavioral neuroscience literature. This review describes (1) a series of neurological and neuropsychological tests which are effectively used as a first screen for behavioral abnormalities in mutant mice, and (2) a series of specific behavioral paradigms, clustered by category. Included are multiple paradigms for each category, including learning and memory, feeding, analgesia, aggression, anxiety, depression, schizophrenia, and drug abuse models. Examples are given from the experiences of the authors, in applying these experimental designs to transgenic and knockout mice. Extensive references for each behavioral paradigm are provided, to allow new investigators to access the relevant literature on behavioral methodology.

Behavioral neuroscientists are poised for a tremendous contribution to the understanding of genes regulating behavior. Transgenic mice, which have a gene added, and knockout mice, which are missing a gene, have been developed for many important neurotransmitters, receptors, second messengers, transporters, transcription factors, and developmental genes. Rigorous analyses of the behavioral phenotypes of these mice are now needed. To enter the fascinating, rapidly expanding field of behavioral genetics, an experienced behavioral neuroscientist just has to say yes to requests from molecular geneticists, to test a new knockout for its behavioral phenotype.

Guidelines for the analysis of the behavioral phenotypes of new transgenics and knockouts would be helpful. Standard appropriate methods are evolving, for the first approach to testing a new mutant mouse. Our laboratory is fortunate to have gained considerable experience in testing many new transgenic and knockout mice being developed on the National Institutes of Health campus in Bethesda (Barlow *et al.,* 1996; Lijam *et al.,* 1996; Norflus *et al.,* 1996; Sango *et al.,* 1995). Our efforts focus on genes expressed in the brain, particularly in mutant mouse models of neuropsychiatric disorders. Over the past 3 years, testing over a dozen different transgenics and knockouts, we have developed a test battery for analyzing the behavioral phenotypes of mutant mice. The present article describes the tests and observations included in the test battery, including general health, gross neurological function, locomotor activity, measurements of motor abilities, simple evaluations of sensory functions, and tests of higher brain function. Further, specific behavioral paradigms for hypothesis-driven phenotyping are discussed. Multiple paradigms for many classes of behavior are listed in Table 1, with extensive references for each. Caveats are presented, for both the methodological and the interpretational phases of these experiments. Representative examples of our approach to the behavioral phenotyping of new knockouts, which demonstrate the power of the screening procedures are described and referenced.

EXPERIMENTAL DESIGN

Molecular genetics methods for developing transgenic and knockout mice have been extensively described (Campbell and Gold, 1996). The first step

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in the behavioral analysis of a new knockout or transgenic mouse is to obtain sufficient animals for each of the relevant genotypes. Breeding problems often limit the availability of large *N*s of each genotype at similar ages. In general, 10 mice ($N = 10$) of each genotype are needed for most behavioral paradigms. The genotypes required must include homozygous mutants ($-/-$) and wild-type littermate controls ($+/+$). Heterozygote littermates ($+/-$) are important in some cases. Wild-type and heterozygous littermates from the F_1 heterozygote matings are the proper control groups for evaluating the behavioral phenotype of a knockout.

Gender differences can be measured in the first pilot studies with the $-/-$, $+/-$, and $+/+$ littermates. If significant differences are found between males and females within a genotype, then the genders are analyzed as separate treatment groups throughout the behavioral testing. Independent gender analysis will require twice the number of animals, e.g., $N = 10$ for male $-/-$, $N = 10$ for female $-/-$, $N = 10$ for male $+/+$, and $N = 10$ for female $+/+$.

Parental strains can be problematic. Most transgenic and knockout mice are currently being developed using substrains of the 129 inbred strain for the embryonic stem cell line, and the C57BL/6J inbred strain for blastocysts and for subsequent breeding. The choice of these two strains is historical and based on technical successes. However, the choice of these two strains is unfortunate for many genes linked to behavioral phenotypes, because of unusual behaviors described in these two strains (discussed in Crawley, 1996; Crawley *et al.,* in press; Gerlai, 1966). For example, some 129 substrains (e.g., 129/J and 129/SvJ) have an incomplete or missing corpus callosum (Livy and Wahlsten, 1991) and perform poorly on learning and memory tasks (Crawley *et al.*, in press). C57BL/6J mice show an unusual propensity to self-administer drugs of abuse, including alcohol (Phillips and Crabbe, 1991) and cocaine (Grahame *et al.*, 1995). Aberrant traits linked to unusual alleles in the background genes of a progenitor strain can raise serious difficulties in interpreting behavioral phenotypes of the mutants. Further, different breeding strategies will result in mutants with different percentages of genetic load from the 129 parent and the C57BL/6J parent. There is no simple solution to this problem, although it has been extensively discussed (Crawley, 1996; Crawley *et al.*, in press; Gerlai, 1966). It may be helpful in some cases to conduct preliminary tests of both parental strains, to calibrate each behavioral paradigm, before beginning the testing of the $-/-$, $+/-$, and $+/+$ littermates on the behavioral paradigms. The best approach is to create congenics. The null mutation is systematically backcrossed into one strain, e.g., the knockouts are bred for several generations back into C57BL/6J, or back into 129/SvEv, to obtain a more homogenous genetic background (Smithies and Maeda, 1995). In addition, new embryonic stem cell lines may become available from inbred strains more commonly used for behavioral research, e.g., a C57BL/6J embryonic stem cell line (Wiles and Keller, 1991; Lederman and Burki, 1991). Further, in studying the behavioral neuroendocrinology genes relevant to reproductive behaviors, cross-fostering to wild-type dams is useful, to allow the investigator to distinguish phenotypes resulting from unusual maternal behaviors of $-/-$ dams, versus unusual behavioral phenotypes intrinsic to the pups.

CONVERTING RAT PARADIGMS TO MOUSE PARADIGMS

Rodents have many general properties in common. However, there are many species-specific behaviors and many behavioral differences between the genus *Rattus* and the genus *Mus.* Some behavioral paradigms can be used identically for mice and rats. For example, photocell-equipped automated open-field equipment detects both mouse and rat movements with approximately equal precision. In other cases, it is sufficient to scale down the equipment from rat size to mouse size, with minor modifications. As examples, rotarod equipment can be converted from rat-size to mouse-size by switching the rotating cylinder to a smaller diameter rod. Mouse operant chambers are commercially available that are smaller in size, have more sensitive levers, and deliver smaller food pellets for reinforcement. Mouse elevated plus mazes are simply smaller versions of rat elevated plus mazes.

However, in many cases, the innate behavioral repertoires of rats and mice differ in ways that preclude adapting a rat behavioral paradigm for use in mice. For example, mice are generally much more active and exploratory than rats. Operant tasks that require suppression of spontaneous behaviors may be more difficult or impossible for mice to perform. Conversely, some tasks are more suitable for mice, e.g., the light ↔ dark anxiety model is more successfully used in mice

than in rats, based on the higher level of exploratory locomotion exhibited by mice (Crawley, 1981, 1989). Differences in sensory, motor, and cognitive functions between rats and mice can be critical, for example, on taste, ability to swim, and spatial learning strategies. To address the species issue, each new behavioral paradigm must be fully evaluated for its applicability to mice. Insight into innate behavioral characteristics of both species may allow the adaptation of some of the standard rat paradigms for mice. New paradigms developed specifically for mice will need extensive validation, using both background strains and the wild-type littermate controls for the null mutation.

THE NEUROLOGICAL AND NEUROPSYCHOLOGICAL TEST BATTERY

The first step in evaluating a new transgenic or knockout mouse is to look for any gross abnormalities that will obviously interfere with further behavioral testing. If the mutants are sick, as evidenced by labored breathing, blood crusted around the nose, poor grooming, very low body weight, and/or other easily observed symptoms, their performance on behavioral tasks is likely to be compromised. Traits that may be caused by illnesses include hypoactivity, hypersensitivity to handling, and/or aggression. A mutation that affects bone or muscle may result in mice that can have difficulties performing tasks that require locomotion or swimming. Blind or deaf mice will not perform on tasks that require visual or auditory cue perception and discrimination. Our laboratory has developed a battery of simple behavioral tests, described below, to evaluate general health, normal reflexes on standard neurological tests, simple locomotor functions, basic sensory processes, and cognition. Test batteries for analyzing the behavioral phenotypes of transgenic/knockout mice are becoming important and useful tools, as evidenced by their development in many laboratories (e.g., Rogers *et al.*, 1996).

Indices of general health are obtained by measuring body weight and rectal temperature and recording observations on any abnormal physical features. These may include poorly groomed fur, bald patches in the coat, or an absence of whiskers, which may indicate unusual home cage behaviors (Hauschka, 1952; Lijam *et al.*, 1996; Long, 1972).

Gross neurologic function is assessed next, using a subset of tests from the screen described by Irwin (1968). These observations and tests are useful for detecting severe neurological dysfunction. The mouse is placed in an empty cage for 3 min, and the presence of abnormal spontaneous behaviors are recorded. These may include wild-running, excessive grooming, freezing, and hunched body posture when walking. Next, the response of the animal to an approaching object, e.g., a cotton-tip swab, is recorded. Most mice will sniff or approach the object, but will then turn away and avoid the object. An abnormal response would be to attack the object or to show no response. The animal is then placed in the center of a platform for analysis of visual cliff behavior. The latency to move to the edge and the number of times the mouse reaches its head over the edge are recorded. Most mice move from the center to the edge in less than 10 sec, and they poke their head over the edge several times in a minute. Mice that have some kinds of sensory deficits, or difficulty controlling movement, cannot perceive the presence of an edge, or are hyperactive, and may walk off the edge.

Several reflexes are then measured. The animal is placed in an empty cage and the cage is rapidly moved from side to side and then up and down. The normal postural reflex is to extend all four legs, in order to maintain an upright, balanced position. The righting reflex is used to further measure the postural reflex. The animal is turned on its back, and the time to right itself to an upright position is recorded. Normal mice will immediately right themselves. The eye blink reflex and ear twitch reflex are measured by simply touching the eye and the tip of the ear, respectively, with a cotton-tip swab. The whisker-orienting reflex is measured by lightly touching the whiskers of a freely moving animal. Since the whiskers of a mouse are continually moving, the whiskers of a normal mouse will stop moving when touched. In many cases, the mouse will turn its head to the side on which the whiskers were touched. To determine visual response to light, the constriction and dilation reflex of the pupil is measured. In a dim room, a penlight or a small flashlight beam is directed at the eye and the constriction and susequent dilation of the pupil are observed when the light is removed. The presence or the absence of reflex responses is recorded, including pupil constriction/dilation reflex, positional eye blink, ear twitch, and whisker-orienting reflex.

Locomotor activity is evaluated by placing the animal in an automated Digiscan open-field arena under standard room lighting. The primary variables used are (1) horizontal activity and (2) total distance, which quanti-

tate overall locomotion, (3) vertical activity, which quantitates rearings, and (4) center distance. Differences in the ratio of center distance to total distance can be used as a preliminary indicator of anxiety, as highly anxious mice tend to avoid the center of an open field (Archer, 1973; Crawley, 1989; Crawley *et al.*, in press). Overall activity will tend to decrease over time, a measure of habituation to the novelty of the open field. Habituation is quantitated by recording activity in 2-min intervals for 60 min.

Motor coordination, balance, and ataxia are tested with the rotarod and the hindpaw footprint tests. The rotarod measures the ability of a mouse to maintain balance on a rotating rod. Two methods have been used: (1) Each mouse is placed on the rotating rod, and the time to fall off is measured, up to a 60-sec maximum. (2) An accelerating rotarod allows the rotation speed to be increased from 4 to 40 revolutions per minute, over a 5-min period. In the accelerating rotarod test, the latency and rotation speed at which the animal falls off are recorded. Performance generally improves over trials; therefore mice are tested twice, with an hour rest period between each trial. The difficulty of the rotarod task can be increased by having the mouse start a trial with the rod rotating at a moderate to high speed, e.g., 20–40 rpm. Conversely, mice with severe motor coordination problems, e.g., due to abnormalities in the cerebellum, will have difficulty remaining on the rotating drum even at very low speeds (Barlow *et al.*, 1996; Sango *et al.*, 1995). The hindpaw footprint test evaluates the walking pattern of mice. Hindpaws are dipped in ink. The subject is then placed at one end of a dark tunnel, $9.2 \times 6.3 \times 35.5$ cm. The footprints are recorded on a clean sheet of white paper that is placed on the floor of the tunnel. The average distance between each stride and the stride variability are measured. Stride variability is calculated from the difference between the longest and the shortest stride lengths. Ataxic mice have shorter stride lengths and strides that are more variable (Barlow *et al.*, 1996).

The acoustic startle response is used to evaluate auditory reflexes. The startle response to a range of sound levels (70 to 120 dB) is evaluated in a sound attenuated chamber. Both the maximum startle response and the threshold for a response are measured (Paylor and Crawley, in press).

Deficits detected on primary measures of health, locomotion, reflexes, motor, and sensory functions may have profound implications. The deficit may, in fact, define a highly relevant phenotype for the gene of interest. For instance, *Atm* knockout mice illustrate the power of simple motor tasks, such as footprint patterns, in analyzing aberrant gait in this animal model of ataxia telangiectasia (Barlow *et al.*, 1996), a human genetic disorder characterized by ataxic gait. In such cases, the primary screen may be sufficient for identifying the relevant behavioral phenotype. Further elaborations of the deficit can then be pursued, where appropriate. For example, if an animal does not show an acoustic startle response in the primary screen, this may indicate that the animal has a hearing impairment or is deaf. When the gene is relevant to auditory acuity, a second stage of testing may then include more sophisticated neurophysiological analyses of auditory threshold, decibel range of hearing, and frequency range of hearing. As another example, if an animal does not show normal passive avoidance behavior, this may indicate that the animal has a visual impairment or is blind, or that the animal has an elevated pain threshold. The second stage of testing may then include more sophisticated tasks, using visual discriminations to assess visual acuity, and extensive analgesia testing to quantitate pain threshold.

In some cases, a deficit on gross neurological, motor, sensory, or health measures will preclude any further behavioral testing on complex behavioral paradigms relevant to the gene of interest. Locomotion and limb movements are required for the performance of almost all behavioral paradigms. A mouse that cannot swim quickly and accurately in the Morris water task will be inaccurately interpreted as having a severe deficit in learning and memory. Deficits in the sense of smell will interfere with many behaviors in mice, a species which uses olfactory cues in exploration, feeding, social interactions, aggressive encounters, sexual behaviors, parental behaviors, etc.. Animals with elevated pain thresholds may perform poorly on negatively reinforced operant tasks involving mild footshock.

However, in many cases, creative choices of appropriate behavioral paradigms can prevent or minimize the problems. For example, a mouse that cannot swim well enough to perform the Morris water task may be able to perform a two-lever task in a small operant chamber or can be evaluated on a contextual fear-conditioned response, both of which require minimal locomotion. Mice with moderate motor impairments can be tested in some types of tasks. For example, γ-protein kinase C (γ-PKC) knockout mice have motor impairments (Abeliovich *et al.*, 1993; Chen *et al.*, 1995), yet they swim as proficiently as their wild-type littermates. Therefore, it was possible to evaluate γ-PKC knockout mice on at least two types of learning and memory tasks (Abeliovich *et al.*, 1993).

Similarly, DBA/2 mice have an age-related hearing impairment (Erway, 1993) and show a poor acoustic startle response (Marks *et al.*, 1989; Paylor and Crawley, in press). These two pieces of information suggest that using tests which require audition would not be advisable in these mice. However, in the prepulse inhibition paradigm, the acoustic startle response in DBA/2 mice is modified by auditory prepulse stimuli which were close to background levels, indicating that normal sensorimotor gating was measurable in mice with poor acoustic function (Paylor and Crawley, in press). Knockout mice missing the gene for a peptide which stimulates feeding may have low body weights, but can be effectively studied with appetite-stimulating drug challenges and nutrient-specific diets. A thorough evaluation of gross deficits, using the proposed primary test battery, will allow the investigator to design appropriate and effective specific tests for complex behaviors linked to the mutated gene. If their sensory or motor impairments are not severe and debilitating, it may be feasible to test knockout and transgenic mice on some types of paradigms for complex behaviors relevant to higher brain function.

Our primary test battery currently includes several additional behavioral tests of general interest. These focus on complex behavioral traits involving higher brain function. Prepulse inhibition is employed to evaluate sensorimotor gating (Braff and Geyer, 1990; Paylor and Crawley, in press). The prepulse inhibition paradigm quantitates the normal suppression of a startle response by a preceding weak, nonstartling prestimulus. Learning and memory processes are evaluated on at least one task, including the Morris water task (Morris, 1981), passive avoidance (Mathis *et al.*, 1994a,b), conditional alternation in a water T-maze (Paylor *et al.*, 1993), and/or cued and contextual conditioned fear (Paylor *et al.*, 1994). Further, the ontogeny of learning and memory (Paylor *et al.*, in press) and the ontogeny of behavioral reflexes (Fox, 1965; Roubertoux *et al.*, 1992) can be evaluated through the test battery, for mutations relevant to developmental processes. Extensive descriptions of the methods for each of these tasks are contained in the publications referenced in Table 1.

THE CONSTELLATION OF COMPLEX BEHAVIORAL PARADIGMS FOR EVALUATION OF HIGHER BRAIN FUNCTION

Transgenics and knockouts are usually developed to test a specific hypothesis about the function of a given gene. The scientific aims fall into three categories: (1) animal models of human genetic disorders; (2) extending knowledge of a gene when the function of the gene product is already known; (3) exploration of the function of a newly discovered gene (or exploration of the function of a gene in mammals after the function of the gene is described in another species, such as *Drosophila*). Rationales for the choice of behavioral paradigms differ somewhat for these three approaches.

Mutant Mouse Models of Human Genetic Diseases

Design of specific behavioral paradigms is dictated by the symptoms of the human disease. A thorough reading of the human clinical literature will yield good ideas about symptoms to model, in mouse knockout and transgenic models of human genetic disorders. On-site observation of patients with characteristic symptoms of the genetic disorder can be extremely useful.

For each individual mutant model of a human genetic disorder, the behavioral neuroscientist can design a highly individualized set of behavioral paradigms. The rich literature from the distinguished fields of ethology and experimental psychology offers many animal behavioral paradigms relevant to the hypothesized function of a specific gene. Good reference sources include major textbooks and review articles (Becker *et al.*, 1992; Crawley, 1985, 1989, in press; Crawley *et al.*, in press; Iversen and Iversen, 1981; Koob *et al.*, 1989; Seiden and Balster, 1985; Willner, 1995).

The goal of mutant mouse models of human genetic disease is often to develop a treatment for the disease. A robust phenotype for the mutant is needed to test the efficacy of proposed genetic and pharmacological therapies. Successful behavioral phenotyping will identify one or two major behavioral abnormalities that can be used as reliable screens. Simple, reproducible, quantitative, automated paradigms are ideal. Defining a simple behavioral phenotype for a knockout can lead directly to gene therapy approaches, as seen in the rotarod performance deficits of *hexB* knockout mice, a model of Tay-Sachs disease (Sango *et al.*, 1995). Embryonic stem cell transplants are now being attempted on these knockouts, with improvement in rotarod scores as the primary behavioral index of functional outcome (Norflus *et al.*, 1996).

Evaluating the Functional Outcome of a Missing or Overexpressed Gene, when the Function of the Gene Product Is Known

Neurotransmitters, receptors, transporters, effectors, synthetic enzymes, and metabolic enzymes have been

TABLE 1
Constellations of Specific Behavioral Paradigms for Hypothesis-Driven Behavioral Analysis of Transgenic and Knockout Mice

Category	Paradigms	References
Learning and memory	Morris water task	Baxter *et al.* (1995)
		Crawley *et al.* (submitted for publication)
		Gage *et al.* (1984)
		Hagan *et al.* (1983)
		Hsiao *et al.* (1996)
		Logue *et al.* (in press)
		Morris (1981)
		Morris *et al.* (1982)
		Murray and Fibiger (1986)
		Page *et al.* (1991)
		Quirion *et al.* (1995)
		Riekkinen and Riekkinen (1995)
		Silva *et al.* (1992)
		Sutherland *et al.* (1982)
		Upchurch and Wehner (1988)
		Wenk (in press)
	Radial maze	Schwegler *et al.* (1996)
		Ammassari-Teule *et al.* (1993)
		Crusio *et al.* (1987)
		Gallagher *et al.* (1983)
		Kesner and Novak (1982)
		Levy *et al.* (1983)
		Olton and Samuelson (1976)
		Reinstein *et al.* (1983)
		Wenk *et al.* (1986)
	Y-maze	Brioni and McGaugh (1988)
		Hsiao *et al.* (1996)
		Mathis *et al.* (1991)
	T-maze	Bertholet and Cruscio (1991)
		Givens *et al.* (1992)
		Henderson (1972)
		Paylor *et al.* (1993)
		Wenk *et al.* (1984)
	Passive avoidance	Brioni and McGaugh (1988)
		Mathis *et al.* (1994a,b)
		Page *et al.* (1991)
		Riekkinen and Riekkinen (1995)
		Santucci *et al.* (1989)
		Sarter *et al.* (1992)
		Wahlsten (1972)
	Active avoidance	Bovet *et al.* (1969)
		Oliverio *et al.* (1972)
		Oliverio *et al.* (1973)
		Peeler *et al.* (1995)
		Royce *et al.* (1971)
	Delayed nonmatching to position	Dawson *et al.* (1994)
		Dunnett (1985)
		Robinson and Crawley (1994)
	Cued and contexual conditioning	Bourtchuladze *et al.* (1994)
		Kim and Faneslow (1992)
		Paylor *et al.* (1994)
		Phillips and LeDoux (1992)
	Eyeblink conditioning	Aiba (1994)
		Chen *et al.* (1996)
		McCormick and Thompson (1984)

TABLE 1—*Continued*

Category	Paradigms	References
Feeding	24-hr consumption	Erickson *et al.* (1996) Smith *et al.* 1994
	Two-choice	Corwin *et al.* (1995) Lucas *et al.* (1989) Rolls *et al.* (1983)
	Macronutrient selection	Bray (1992) Tempel and Leibowitz (1990)
	Limited access	Cooper and Higgs (1994) Crawley *et al.* (1990) Flanagan *et al.* (1992) Salorio *et al.* (1994)
	Sham feeding	Davis and Campbell (1973) Young *et al.* (1974)
Pain / analgesia	Tail-flick	Dewey *et al.* (1969) Dourish *et al.* (1990) Pick (1996) Pick *et al.* (1991)
	Hot-plate	Fields *et al.* (1991) Rubinstein *et al.* (1996) Wiesenfeld-Hallin *et al.* (1990)
	Stress-induced analgesia	Lewis *et al.* (1980) Madden *et al.* (1977) Menendez *et al.* (1993) Seeger *et al.* (1984) Vanderah *et al.* (1992)
Aggression	Resident–intruder	Jones and Brain (1987) Maxson (1992) Miczek *et al.* (1994) Nelson *et al.* (1995)
	Isolation-induced	Crawley *et al.* (1975) Giacalone *et al.* (1968) Miczek *et al.* (1994) Sandnabba (1996) Sandou *et al.* (1994) Ewalds-Kvist and Selander (1996)
Reproductive	Sexual behaviors	Rissman *et al.* (1997) Nishimori *et al.* (1996) Lydon *et al.* (1995) Mosig and Dewsbury (1976) McGill (1962)
	Parental behaviors	Nishimori *et al.* (1996) Brown *et al.* (1996) Cohen-Salmon *et al.* (1985)
Anxiety models	Elevated plus maze	Flint *et al.* (1995) Lister (1987) Stenzel-Poore *et al.* (1994) Stinchcomb *et al.* (1989) Trullas and Skolnick (1993)
	Light ↔ dark exploration	Crawley (1981) Hughes *et al.* (1990) Mathis *et al.* (1995) Pierrefiche *et al.* (1993)
	Social interaction	File (1980) File and Andrews (1994) Hahn and Schanz (1996)
	Footshock-induced freezing	Holmes *et al.* (1994) Kalin *et al.* (1988)
	Conflict test	Commissaris *et al.* (1986) Koob *et al.* (1986) Vogel *et al.* (1971)

TABLE 1—*Continued*

Category	Paradigms	References
Depression models	Porsolt swim immobility	Redrobe *et al.* (1996) Hernando *et al.* (1996) Drugan *et al.* (1989) Hilakivi and Lister (1990) Nikulina *et al.* (1991) Porsolt *et al.* (1977) Willner (1995)
	Learned helplessness	Drugan *et al.* (1989) Maier *et al.* (1982) Seligman and Maier (1967) Sherman *et al.* (1979)
Schizophrenia models	Prepulse inhibition	Geyer *et al.* (1990) Hoffman *et al.* (1993) Paylor and Crawley (in press)
	Latent inhibition	Gray *et al.* (1995) Weiner *et al.* (1987)
	Chronic amphetamine	Alexander *et al* (1996) Ellison (1994) Segal *et al.* (1981)
Drug abuse models	Self-administration / voluntary consumption	Belknap *et al.* (1993) Berrettini *et al.* (1994) Chu and Kelley (1992) Devine and Wise (1994) Grahame *et al.* (1995) Phillips *et al.* (1994) Piazza *et al.* (1991) Seale (1991)
	Intracranial self-stimulation	Bozarth and Wise (1984) Esposito and Kornetsky (1978) Goeders and Smith (1983)
	Place conditioning	Menkens *et al.* (1992) Miner (in press) Shippenberg and Heidbreder (1995)
	Sensitization	Kalivas *et al.* (1985) Kuczenski and Segal (1988) Tolliver *et al.* (1994)
	Tolerance	Crabbe *et al.* (1993) Collins *et al.* (1988) Marks *et al.* (1983)
	Withdrawal	Koob *et al.* (1992) Rasmussen *et al.* (1990) Way *et al.* (1969)

elucidated for many neuroanatomical pathways, physiological actions, and behavioral functions. Transgenic technology and knockout technology make it possible to test hypotheses about the importance of the gene for the neurotransmitter, receptor, etc., when the gene is missing or overexpressed from the earliest stages of development. Specific behavioral functions are analyzed, based on the established literature for a specific neurochemical.

Guidelines for choosing behavioral paradigms to test a specific behavioral function in transgenic and knockout mice include several elements. Multiple distinct behavioral paradigms should be employed to evaluate each function. Further, it is a good idea to choose the most standard and well-characterized paradigms. Behavioral neuroscientists will want to compare knockout results with the existing literature, and important findings will need to be replicated by other laboratories.

Finally, rigorous statistical analyses are needed; paradigms that are amenable to strict statistical tests are preferred.

Table 1 offers suggestions on constellations of multiple paradigms for a variety of categories of tasks relevant to behavioral neuroscience. For each paradigm, recent references are listed which describe the methods and show the expected types of results. Earlier literature on behavioral tests for genetically defined mice is reviewed in Sprott and Staats (1975).

References listed in Table 1 provide many examples of the successful behavioral phenotying of transgenic and knockout mice, including mouse models of human genetic disorders (Barlow *et al.,* 1996; Hsiao *et al.,* 1996; Sango *et al.,* 1995; and reviewed in Lee *et al.,* 1996), and mutations of genes with known functions (Abeliovich *et al.,* 1993; Aiba *et al.,* 1994; Bourtchuladze *et al.,* 1994; Brown *et al.,* 1996; Chen *et al.,* 1995; Nelson *et al.,* 1995; Sandou *et al.,* 1994; Silva *et al.,* 1992; Stenzel-Poore *et al.,* 1994). There are several important publications of the application of these multiple behavioral paradigms to the behavioral phenotyping of knockouts relevant to behavioral neuroendocrinology. Estrogen receptor knockout mice were dramatically impaired in fertility (Lubahn *et al.,* 1993). Female estrogen receptor knockout mice did not display sexual receptivity, although their attractiveness to males was normal (Rissman *et al.,* 1997). Male estrogen receptor knockout mice showed increased aggressive behaviors (Ogawa *et al.,* 1996). Knockout of the progesterone receptor produced female mice that did not display lordosis behavior after estrogen priming (Lydon *et al.,* 1995). Oxytocin knockout females demonstrated normal maternal behavior but an inability to nurse pups, due to a deficit in milk ejection (Nishimori *et al.,* 1996). Transgenic mice overexpressing corticotropin releasing factor showed elevated plasma levels of ACTH and glucocorticoids, in conjunction with anxiogenic behaviors on the elevated plus maze (Stenzel-Poore, 1994).

Exploring the Functions of New Genes

In the unforgettable words of Ogden Nash (1980):

> ''. . . If called by a panther,
> Don't anther.''

If called by a molecular geneticist to test a knockout for a newly discovered gene expressed in the brain, with no *a priori* hypothesis about the function of the gene, the behavioral neuroscientist may be tempted to not answer. One wonders where to start and how far to go. On the other hand, the first phenotypic characterization for a new gene is an exciting opportunity for a unique discovery.

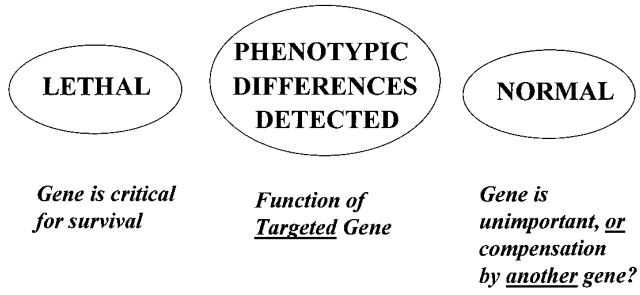

FIG. 1. Knockout mice: Potential outcomes.

We approach this conundrum by employing the tests described above, for the discovery of behavioral phenotypes for new genes. The primary battery will yield considerable information about basic physiological and behavioral functions and may lead directly to more specific hypotheses. Constellations of complex behavioral paradigms can be then be chosen, e.g., multiple tests from each of several categories of behavior, to evaluate higher brain function (see Table 1). Anatomical sites where the gene is expressed can guide the choice of specific paradigms. A gene expressed primarily in the hippocampus can be tested on learning and memory tasks and prepulse inhibition; a gene expressed primarily in the hypothalamus can be tested on feeding, stress paradigms, sexual behavior, and parental behavior. In an example from our laboratory, knockouts of the gene *Dvl-1,* a developmental gene known from *Drosophila* and recently found to be expressed in the mouse hippocampus, show specific deficits in acoustic startle and prepulse inhibition, illustrating the discovery of interesting and unexpected functions for a newly described gene (Lijam *et al.,* 1996).

CONCLUSIONS

Three outcomes are possible in behavioral phenotyping of transgenic and knockout mice (Fig. 1). (1) The gene may be so important that the mutation is lethal. Mutants do not live long enough for behavioral testing. (2) Mutation of the gene may produce an interesting and important behavioral phenotype. These are the success stories, defining the characteristics that reflect the function of the gene of interest. (3) Mutation of the gene

may produce no detectable behavioral phenotype. The gene may truly be unimportant with respect to the functions tested. Alternatively, the lack of significant phenotypic differences may reflect compensation for the missing gene by another gene during development. Compensatory processes are a fascinating phenomenon, relevant to the developmental biology of the brain. For example, appetite appears to be redundantly regulated by several monoamines and neuropeptides in the hypothalamus. It will be very interesting to find out which genes are upregulated, in cases where one of the regulatory peptides is knocked out but feeding behaviors remain normal. However, investigations of complex developmental neurobiology may be beyond the expertise or interest of the behavioral neuroscientist.

When no behavioral phenotype is detected, after a reasonably rigorous analysis, the experiment is generally considered negative and concluded. Conversely, when an interesting behavioral phenotype is detected, the behavioral neuroscientist may choose to invest more of the laboratory's efforts in further in-depth investigations. Conditional knockouts, expressed in a specific brain region and/or induced at a specific stage of development, may increase the attractiveness of the knockout technology to behavioral neuroscientists.

Collaborations between behavioral neuroscientists and molecular geneticists represent a paradigm shift in studying brain and behavior. Questions of authorship, funding, and proprietary rights to mutant mice may arise. These issues will be addressed over the next years, by institutions, funding agencies, and reasonable scientists throughout the world. In our experience, the efforts required to begin working with transgenic and knockout mice are trivial, compared to the remarkable intellectual challenge they offer and their therapeutic potential for treating human genetic diseases.

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