

Interactive report

Behavioral phenotyping of transgenic and knockout mice:
experimental design and evaluation of general health, sensory functions,
motor abilities, and specific behavioral tests¹

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Abstract

Rigorous experimental design can minimize the high risk of false positives and false negatives in the behavioral phenotyping of a new transgenic or knockout mouse. Use of well established, quantitative, reproducible behavioral tasks, appropriate Ns, correct statistical methods, consideration of background genes contributed by the parental strains, and attention to litter and gender issues, will maximize meaningful comparisons of $-/-$, $+/-$, and $+/+$ genotypes. Strategies developed and used by our laboratory are described in this review. Preliminary observations evaluate general health and neurological reflexes. Sensory abilities and motor functions are extensively quantitated. Specific tests include observations of home cage behaviors, body weight, body temperature, appearance of the fur and whiskers, righting reflex, acoustic startle, eye blink, pupil constriction, vibrissa reflex, piano reflex, Digiscan open field locomotion, rotarod motor coordination, hanging wire, footprint pathway, visual cliff, auditory threshold, pain threshold, and olfactory acuity. Hypothesis testing then focuses on at least three well-validated tasks within each relevant behavioral domain. Specific tests for mice are described herein for the domains of learning and memory, feeding, nociception, and behaviors relevant to discrete symptoms of human anxiety, depression, schizophrenia, and drug addiction. An example of our approach is illustrated in the behavioral phenotyping of C/TAPO knockout mice, which appear to be normal on general health, neurological reflexes, sensory and motor tasks, and the Morris water task, but show remarkably enhanced performance on contextual fear conditioning. © 1999 Elsevier Science B.V. All rights reserved.

1. Introduction

Targeted gene mutation technology represents a powerful new tool for biomedical research. When the targeted gene is expressed in the brain, the behavioral phenotype of the mutant mice may reveal genetic mechanisms underlying normal behaviors, and may increase our knowledge of genetic factors in neuropsychiatric disorders. *Transgenic mice* have a new gene, or an additional copy of an existing gene, added to the genome. *Knockout mice* have a targeted gene deletion, such that no product of the mutated gene is synthesized in the null mutants. The methods for develop-

ing the mutation, and breeding strategies to generate null mutants in the F2 and subsequent generations, are extensively described in this volume and elsewhere [11,54,76].

Approximately 100 different genes expressed in the central nervous system have been targeted and phenotyped in transgenic and knockout mice to date [4,11,52,54,68]. Reported behavioral phenotypes include aberrant social, reproductive, and parental behaviors, aggression, feeding disorders, learning and memory impairments, anxiety-like behaviors, and altered responses to antidepressants, antipsychotics, ethanol, and psychostimulant drugs of abuse. Experimental design is presently being optimized for thorough evaluation of behavioral phenotyping in mutant mice. This review is designed to suggest general methods that have been validated in our laboratory and others. Specific protocols for individual behavioral tasks can be found in the original publications referenced throughout the text, and in several recent reviews [11,16,19,20,34,38,54,74].

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1. The mice

After the mutation has been successfully introduced, and the gene product shown to be correctly overexpressed or absent, the first elements are mated with wildtype mice. A Mendelian distribution of 1:2 offspring is predicted. The strain of mouse used for the embryonic stem cells, for the donor blastulas, and for the breeding, can greatly affect the behavioral phenotype. For example, some 129 substrains (e.g. 129/J and 129/Sv) have an incomplete or missing corpus callosum [42], and perform poorly on learning and memory tasks [16,76]. Background genes that are randomly contributed by each parental strain often interact with the mutated gene of interest, creating false positives [2,15,16,26,76]. For example, human Alzheimer's mutant β -amyloid precursor protein overexpressed in C57B6 lelelet mice produces amyloid plaques in the brain and poor learning and memory on the Morris water task [31]. The same transgenic insertion in FVB/N mice did not show plaque formation and was lethal at too young an age for learning and memory testing. No single strain can solve all of the potential breeding problems. For some experiments, breeding into the 129/Sv strain is a good solution to unify the background genes by using the same strain for the embryonic stem cells and the parental breeders [39]. Other experiments benefit from a congenic breeding strategy into C57BL/6J, a standard, commercially available inbred strain that shows intermediate values on most behavioral phenotypes. This strain has been characterized on a large number of behavioral tasks and is a reasonably prolific breeder [2,16,69].

Numbers of animals for standardized experimental designs and appropriate statistical tests are a minimum of $N = 10$ - / - null mutants, $N = 10$ + / - heterozygotes and $N = 10$ + / + wildtype littermate controls. If a gender effect is detected, $N = 10$ of each gender and each genotype is required. Ages of the animals are approximately equivalent across genotypes in accordance with the goals of the experiment. For example, adult mice are best tested between ages 3 and 8 months; aged mice between 12 and 18 months; juvenile mice between 2 and 6 weeks.

1. Preliminary observations

A series of carefully conducted preliminary observations of general health, home cage behaviors, sensory abilities, and motor functions is first conducted for each mouse to avoid spurious false positives. If an animal has a major health problem or a gross motor defect it will be unable to perform many behavioral tasks for reasons not necessarily specific to the mutation. If an animal is blind or deaf, specific behavioral tests can be designed around the sensory deficit, such as olfactory learning tasks for blind mice or tactile startle tasks for deaf mice.

Our laboratory developed a set of preliminary observations and neurological reflexes which we use to assess

gross defects in mutant mice [20]. This first step is likened to 'giving your mouse a physical exam.' The mouse is weighed, its body temperature is taken, and the appearance of its fur and whiskers is noted. Home cage locomotion, grooming, nesting, sleeping, and fighting patterns are recorded. Neurological reflexes are tested in each mouse. These include eye blink, ear twitch, whisker twitch, and righting reflex.

We next evaluate motor functions. Each mouse is tested for normal exploratory locomotion on the Dergiscan open field [56]. The mouse is placed in the photocell equipped automated open field box for a 5 min test session during which the Accuscan software calculates total distance traversed, number of movements, horizontal activity, vertical activity, and center/perimeter time. Each mouse is next tested for motor coordination on the Hasle automated accelerating rotarod [33,67]. The mouse is placed on the cylinder and the speed of the cylinder rotation is gradually accelerated from 4 to 40 revolutions per min over a 5-min period. Latency to fall from the rotarod is recorded. The fall is approximately 6 inches, a height that mice can easily fall and land on their feet without injury. Neuromuscular strength is tested by the wire hang test [60]. The mouse is placed on a wire cage lid and the lid is gently waved in the air so the mouse grips the wire. The lid is then turned upside down, approximately 6 inches above the surface of soft bedding material. Latency to fall onto the bedding is recorded, with a 60 s cut-off time. Gait is measured with the footprint test [3]. The two hindpaws are dipped into black ink. The mouse is then immediately placed onto white paper in a dark narrow tunnel approximately 12 inches long. The ink is then wiped off the feet and the pattern and pathway of the black footprints on the white paper are calculated.

Sensory functions are then assessed. Visual ability is measured in the visual cliff test conducted on a box with a ledge. The inner surface of the box and ledge are covered with black and white checkered contact paper which emphasizes the ledge drop-off. A piece of clear plexiglas spans the ledge so that there is no actual drop off, just the visual appearance of a cliff. Normal mice will stop at the 'edge' and explore the plexiglas flume before walking forward. Blind mice will not see the appearance of the edge and will walk forward across the plexiglas immediately. Hearing is assessed with the acoustic startle test [59]. The reflexive flinch and eyeblink to a sudden loud noise comprise the standard acoustic startle response. An automated startle system is used to deliver the startle stimulus and measure the response of the mouse. The mouse is placed in a small cylindrical restraining tube within a sound-attenuating chamber. Stimulus tones are varied randomly from 70 dB to 120 dB, 40 msec duration, in the presence of background white noise at 70 dB. A standard battery of tones begins at threshold sound levels of 72 and 75 dB, includes moderately loud tones of 80, 90, and 100 dB, and ends at the loudest sound levels, 110, 115, and

120 dB. Whole body flinch amplitude is automatically recorded by the pressure transducer. Tactile startle is measured by whole body flinch amplitude to a brief puff of air delivered by the system to an area near the face. A typical session lasts for about 30 min, after which the mouse is returned to the home cage. Sense of touch can be measured with Von Frey hairs, made of thin wires which are used to gently touch the footpads, to measure the reflexive twitch to touch. A simple olfactory test can be used, e.g. latency to locate an odiferous piece of food, e.g. a piece of chocolate smear of peanut butter, buried under the litter in a clean test cage, or time spent sniffing a novel odor such as vanilla extract painted onto the test cage wall.

4. Specific behavioral tasks to test discrete hypotheses

Targeted gene mutation is designed to address specific hypotheses about the behavioral role of a gene. The set of specific behavioral tasks to be used for behavioral phenotyping is designed around the hypothesis. Genes for neuropeptides in the hypothalamus might be analyzed in tests of feeding behaviors, sexual behaviors, and stress-related behaviors. Signalling genes expressed in the hippocampus might be analyzed in tests of spatial learning and memory. Genes for serotonin receptor subtypes might be analyzed in tests of aggression, feeding, and mouse behaviors relevant to symptoms of human depression. A thorough knowledge of the behavioral literature is required to choose the optimal constellation of behavioral tests to address the critical hypotheses.

In some cases, tests designed for rats can be directly used for mice. For example, the Digiscan automated open field works equally well for rats and mice, and the resident/intruder procedure for aggressive behaviors is applicable to males of both species. In some cases, the equipment can be modified for mice often simply by building a smaller version of the rat test apparatus. For example, a mouse rotarod and a mouse elevated plus maze are downsized versions of the rat rotarod and rat elevated plus maze. In some cases, the equipment must be changed to accommodate the behavior of the mouse. For example, lighter weight levers allow a mouse to conduct a lever press task in an operant chamber; alternatively, a nose-poke insert replaces the lever in an operant chamber to accelerate the autoshaping process, because mice tend to explore dark holes more readily than they press levers. In some cases, mouse and rat behaviors are too dissimilar. For example, social interaction is easily measured between two rats placed together in a new environment, but mice will primarily explore the new environment rather than interacting each other. Rats can perform complex operant tasks which appear to be far beyond the abilities of mice.

This article provides an overview of several behavioral tasks that our laboratory and others have validated for mice. The tasks described in this review represent only a

small subset of the applicable behavioral tests available in the wider behavioral neuroscience literature. The reader is referred to the many excellent chapters in this volume which describe multiple tasks in specific behavioral domains in much greater detail.

Because each task has its own limitations, it is best to employ multiple tasks for each behavioral domain of interest to avoid false negatives. For example, a mutation in a gene expressed mainly in the amygdala may produce performance deficits on fear-conditioned learning tasks which require an intact amygdala, but not on spatial navigation learning tasks, which require an intact hippocampus. When sensory or motor deficits are detected during the preliminary screen, the choice of the multiple tasks is limited by the physical disability of the mutants. Knowledgeable behavioral neuroscientists can generally identify two or three good tasks for each type of behavior relevant to each hypothesized function of the gene product.

4.1. Feeding

Twenty-four hour consumption of normal rat chow in the home cage is measured by daily weighing of the food [23,32]. Standard amounts of food are provided by the investigator. Another approach is a challenge test, measuring consumption of a palatable food source or specific macronutrients during a test session [17]. A high carbohydrate/high fat source, such as sweetened condensed milk, cookie mash, or a commercially available powdered chow diet, is provided in a clean test cage. The mouse is placed in the cage for the fixed time period. Alternatively, a two-choice paradigm involves presentation of the special diet in a second food hopper next to the standard chow in an identical food hopper, or presentation of test liquid solutions in separate water bottles, attached to the home cage [5,7]. Food or water restriction may be performed in special circumstances, e.g. to study a gene that regulates consumption only after an overnight fast, or as a necessary component of food or water reinforcement in learning and memory tasks.

4.2. Learning and memory tasks

Morris swim tasks [51] have been standardized to measure spatial navigation learning and memory in mice [16,71,76]. Each mouse learns to swim in a circular pool of water to locate a submerged hidden platform. The water level is 30 cm deep, maintained at 24–26°C with aquarium heaters, and changed daily. Non-toxic Crayola white paint is added to make the water opaque and the platform invisible to the mouse. An automated video tracking software system is used to quantify swim speed, swim pathway, latency to reach the platform, and time spent in each of the four quadrants of the pool. Mice are excellent swimmers and readily dry themselves off and groom when removed from the pool. While additional drying or hair is

usually not necessary, a soft cloth is available to dry the mice and a hot plate is available to place under the cage, if needed. During training on the visible platform task, the platform is raised above the surface of the water, or a prominent cue is attached to the platform to extend above the surface of the water. The mouse is gently guided to the platform by the experimenter and allowed to remain on the platform for 20 s. The mouse is then placed along the edge of the pool, facing the wall, in one of four randomized start locations equidistant around the perimeter. The mouse is given 60 s to swim to the visible platform and allowed to remain on the platform for 20 s. Latency to find the visible platform over four successive trials is recorded. Mice that do not swim to the visible platform are noted, and given further guided training by the experimenter. Any mouse that does not swim, and any mouse that only floats, is eliminated from the experiment. Mice that learn to navigate to the visible platform are then trained on the hidden platform task. The platform is submerged below the surface of the water, so that the mouse cannot see the location of the platform. Each trial begins with the mouse being placed in the pool, facing the wall, in a different quadrant on each trial. Most acquisition training on the hidden platform task consists of two or three blocks a day of four trials per block — 8 to 12 trials per day, for three to five consecutive days. Fewer trials per day may be used for mice that learn rapidly. More days may be needed for mice that learn slowly. After the acquisition curve reaches the performance criteria (usually 14–20 s latency to find the hidden platform), the probe trial is administered. The platform is removed from the pool. The mouse is placed in the pool for a single 60 s trial. Time spent in the trained quadrant, where the platform was previously located, is compared to time spent in the other three quadrants, to confirm acquisition. If time spent in the trained quadrant is significantly greater than time spent in each of the other quadrants, the mouse has learned the location of the hidden platform, rather than having learned an effective general search strategy. The probe trial is generally administered at the end of the last training day. Additional probe trials may be administered on subsequent days, as a measure of retention. Additional training may precede subsequent probe trials. Another approach is to test reversal learning, in which the mouse is retrained with the hidden platform in another quadrant. Probe trial testing is similarly conducted after reversal training.

Cued and contextual conditioning is a fear-conditioning task that measures memory of an aversive experience, and the stimuli present during the aversive experience [10,16,24,37,71,75]. A standard footshock shuttle box, or a specialized automated freeze monitor and software package, is used to control footshock delivery and to measure duration of freezing behavior. Freezing, a standard response to a sudden aversive stimulus, is defined as complete behavioral immobility except for respiration. On day 1, the mouse is placed in a chamber and allowed to explore

for 2 min. The chamber is square and illuminated by normal house lighting. An auditory stimulus (80 dB white noise) is then presented for 30 s. One footshock, 0.5 mA, 2 s duration, is then delivered. The animal is then left in the cage for 30 s, then returned to its home cage. On day 2, the mouse is returned to the same testing chamber. The time spent in freezing behavior is measured over a 5-min period. Total number of seconds spent in complete behavioral immobility are tallied. One hour later the mouse is placed into the same testing chamber, but the context has been changed. The grid floor is now covered with plexiglas, the square is divided by a piece of plexiglas to form a triangle-shaped chamber, a red chamber light is on, and an odor is painted onto the chamber walls, e.g. vanilla or lemon extract. Freezing is quantitated for 3 min. Then the auditory cue is presented. Freezing is quantitated over the next 3 min, in the presence of the 80 dB white noise cue. The mouse is then returned to its home cage. Thus, memory is assessed by measuring freezing under three conditions for each mouse: context, altered context, and auditory cue. Chambers are thoroughly cleaned after each test session.

Maze learning and avoidance tasks are among the oldest learning and memory tasks used in rodents. Passive and active avoidance tasks measure memory of an aversive experience, through simple avoidance of a location in which the aversive experience occurred [8,44,57,67]. A commercially available automated apparatus consists of two connected chambers, one lighted, one dark. Mice tend to prefer a dark environment and will immediately enter the darkened chamber. Passive avoidance is a two day task. On day 1, the training session, the mouse is placed in the lighted chamber for 10 s. The door to the dark chamber is then opened, and latency to enter the dark chamber is measured as a control for visual ability and motor activity. Immediately after the mouse enters the dark chamber, a 0.3 mA, 1 s footshock is delivered. The mouse remains in the dark compartment for 10 s after the shock to allow formation of the association between the dark compartment and the footshock. The mouse is then returned to the home cage. On day 2, the retention test session, the mouse is placed in the lighted compartment and the door is opened. Latency to enter the dark compartment is measured, with a 300 s cut-off time. The mouse is then returned to its home cage. An additional exposure to the single footshock on day 2, followed by latency to enter the dark on day 3, can be used to obtain additional information on acquisition of passive avoidance in mice with apparent prior acquisition. For active avoidance, using the same chamber, with the same parameters, the mouse must move into the opposite chamber to avoid receiving a footshock. Latency to enter the non-shocked chamber is the measure of learning.

Spatial maze tasks include the T-maze, Y maze, radial mazes, and Barnes maze [21,30,58]. Acquisition of location of a terpreter over repeated trials provides the mea-

sure of learning and memory. The Barnes maze is a circular platform, 1.1 meters in diameter, in which 36 holes, each 8 cm in diameter, are equally spaced around the perimeter of the circle, 10 cm from the edge. Mice tend to poke their noses into holes, especially to explore dark holes which may lead to escape routes away from a lighted, open field. One of the holes leads to a dark, enclosed box, located just below the circular platform. The mouse is placed in the center of the platform under a start box. The start box is lifted and the mouse is guided to the escape tunnel where it remains for 1 min. Then the mouse is placed back in the start box and allowed to explore the maze. When the mouse finds the correct hole it escapes into the dark box. The mouse is allowed to remain in the escape box for 1 min then removed from the box and given repeated trials in which it is placed in the center of the platform and allowed to explore for 5 min per trial, 1–4 trials per day, for up to 20 training days. Time to locate the tunnel hole, distance travelled, and number of errors on each training day (noses pokes into the incorrect hole) provide the measures of acquisition.

The T-maze is shaped like the letter T, with either the left or right arm containing concealed cups. One Noyes food pellet or 0.05 ml tap water is used for food or water reinforcement, respectively, in food or water restricted mice, respectively. In the continuous reinforcement procedure, the mouse is placed in the start end of the T, allowed to explore the maze and obtain the reinforcer. The mouse must then return to the start box and choose the opposite arm on the next trial to obtain the reinforcer. Twelve trials per day are conducted for up to 10 consecutive days. Acquisition of the procedure and choice accuracy in this alternation task are the measures of procedural and learning abilities.

Operant tasks are more difficult for mice than for rats, but mice can learn some lever press acquisition tasks [48,64]. Food or water restricted mice are habituated to the operant chamber and shaped to press the lever for the food (small food pellet) or water (0.05 ml tap water) reinforcer, respectively. Number of trials to acquire the lever press task is the simplest measure of learning in mice. The more complex non-matching to position task requires the mouse to receive reinforcement for pressing the opposite lever to the lever illuminated on the previous trial by the cue lamp above the lever. A discriminative go-no go task for mice requires the mouse to press the lever only when the cue light is illuminated to obtain the reinforcement, or to press the lever only during the period in which a tone is sounding. Modifications of the rat operant chamber for mice include levers that are smaller and more sensitive to the touch of the mouse paw, and a module which substitutes the lever for a nose-poke into a dark hole, to obtain the reinforcement.

Motor learning is evaluated by repeated daily testing on the rotarod [60]. Reduction in latency to fall, over repeated testing sessions, provides a measure of cerebellar learning.

4.3. Pain sensitivity tests

The two methods to assess analgesia that have been well validated and standardized for mice are the tail flick and hot plate tasks [22,62,66]. Tail flick nociception measures a simple spinal reflex to a sudden, painful thermal stimulus. A photobeam is used to apply a heat stimulus to the tail. Latency to flick the tail out of the path of the light beam is measured. The photobeam is turned off if the tail is not flicked away within 15 s, to avoid tissue damage.

Hot plate nociception is a similar reflex which requires higher brain centers. The mouse is placed on the surface of a hotplate which is maintained at 50–55°C. A plastic frame encloses the surface so that the mouse cannot jump out. Latency for the mouse to raise and lick its forepaw, or to jump up, is recorded. The mouse is then immediately removed from the hotplate. If the mouse has not responded within 30 s, it is removed from the hotplate, to prevent tissue damage.

Pain sensitivity to footshock is assessed by threshold analysis as a control for procedures requiring a footshock, e.g. some of the learning and memory tasks described above. A sequence of single footshocks is delivered in the avoidance chamber and the mouse is observed for flinching, jumping, running, and vocalization. When these behaviors are observed the sequence is terminated. The sequence begins with 0.075 mA and proceeds through 0.1 mA, 0.15 mA, 0.25 mA, and 0.35 mA, and 0.45 mA single footshocks of 1 s duration each. Most mice show behavioral responses to the lower footshock levels and therefore do not receive the higher footshocks.

4.4. Anxiety-related behaviors

Light ↔ dark exploration is an ethologically-based approach-avoidance conflict test which is sensitive to anxiolytic drug treatments [14,16,18,45]. Mice prefer a dark, enclosed, small space over a brightly lit, open, large space. However, mice are also highly exploratory. The light ↔ dark exploration task represents a naturalistic conflict between the tendency of mice to explore a novel environment versus the tendency of mice to avoid a brightly lit open field. The mouse is placed into the large, lighted compartment of a two-compartment chamber. The mouse repeatedly enters the adjoining small, dark, enclosed chamber, then emerges back into the larger, lit, open chamber. Number of transitions between the two chambers and time spent in each chamber are automatically recorded through a photocell array across the border between the two chambers over a single 10-min test session.

The elevated plus maze task similarly measures the conflict between exploration of a novel environment and avoidance of brightly lit open areas [25,41,72,70]. An additional factor is that the surface is raised 1 meter from the floor. The elevated plus maze is in the shape of a π . Two alternate arms are dark and enclosed, while two

alternate arms are open, lit, and without edges. The mouse is placed in the center of the T and allowed to explore the maze for 5 min. Numbers of entries into the open arms versus number of total arm entries, and time spent on the open arms versus the closed arms, provide the measures of anxiety-related behavior.

The Digiscan open field measure of center time versus perimeter time gives a less specific measure of anxiety-related behavior. In a brightly lit open area, mice will tend to stay near the walls of the open field rather than enter the center region. Habituation to the novelty of the open field, i.e. reduction of perimeter time and increase in the center time, is measured by recording activity in 5 min consecutive intervals over a 1-h period. Repeated 1 h tests over several days gives a further measure of habituation to the aversive properties of the novel open field. While the open field test is not as specific for anxiety-related behaviors, the center time parameter provides some indications that can be used as a starting point for further testing on the more specific anxiety-related tests.

4.3. Depression related behavior

The Porsolt swim test is used to evaluate "behavioral despair," a measure of failure to seek escape from an aversive stimulus [63]. The mouse is placed in a cylinder of room temperature tap water and swimming behavior is observed over a 10-min test session. The water is approximately 12 inches deep, such that the animal cannot balance on its feet or tail. The water surface is approximately 6 inches from the top of the cylinder, such that the animal cannot jump out. Rodents will generally swim. Animals treated with certain drugs or lesions will stop swimming and will float. Floating time is considered the measure of depression-like behaviors, in that the animal has stopped swimming and 'given up' on finding an escape route. Floating time is decreased by treatment with antidepressant drugs. Any mouse that does not swim or float is immediately removed from the water. At the end of the 10 min swim test the mouse is dried with a towel and returned to its home cage.

4.5. Schizophrenia related behavior

Deficits in prepulse inhibition are common in schizophrenic patients and may measure attentional dysfunctions that contribute to auditory hallucinations [27]. Prepulse inhibition is a sensorimotor gating reflex, similarly quantitated in mice, rats, and humans [27,59]. When the startle stimulus is immediately preceded by a milder stimulus, delivered immediately before the startle stimulus, the mouse will flinch less to the startle stimulus. Prepulse tones of 74, 82, or 90 dB are randomly presented 100 msec before each 100 dB or 120 dB startle tone. Whole body flinch amplitude is again automatically recorded. To evalu-

ate a separate sensory modality, a puff of air is used as the prepulse, before the acoustic or tactile startle stimulus.

4.7. Social behaviors

A variety of social behaviors can be quantitated by videotaping the mice over 24 h periods in the home cage [39]. Observations during the night are conducted under red light, which does not disturb the mice. Group huddling while sleeping is one characteristic of normal mouse social behavior. Nest building can be observed over a 1-h period after placing a Nestlet cotton square or other nesting material in the floor of the home cage. Depth of nest provides a quantitative measure of nest building. Fighting can be quantitated from the videotapes by scoring number and duration of attacks.

The tube test for social dominance can be used to evaluate aggressive behaviors [39,40]. Two mice are placed in opposite ends of a plexiglas tube, 3 cm in diameter, 35 cm long. The mouse that advances beyond the midpoint is considered the dominant male. The mouse that backs away from the midpoint is considered the subordinate male. Mice are removed immediately after scoring. Fighting does not occur.

Isolation-induced standard opponent and resident-intruder testing for aggression in male mice is conducted according to established methods [16,49,53]. A standard subordinate test opponent male is placed in the cage of the test male. The test male has been singly housed for approximately one month. Attacks over a 5 min test session by the test male are scored for latency to first attack, number of attacks, number of bites, chasing, and tail-rattling.

4.8. Sexual and reproductive behaviors

Mouse sexual and parental behaviors can be scored using standardized methods [12,55,65,77]. Male sexual behavior is quantitated by observational methods for latencies and frequencies of mounts, intromissions, and ejaculations. Female sexual behavior is quantitated by standardized observations methods for the lordosis response. Pregnancies and number of pups delivered per pregnancy are measures of reproductive success. Lactation can be scored by an observer. Presence of milk in the stomach of the pup can be ascertained in cases of early postnatal death. Parental behaviors are measured by quantitating retrieval of pups to the nest and sitting with the pups in the nest. Before weaning, isolated mouse pups emit ultrasonic vocalizations which alert the parent that the pup is out of the nest and triggers retrieval behavior. Olfactory cues are used by the parents to identify their new pups. Quantitating parental behaviors provides a good example of the need to test sensory abilities before conducting a specific test. If sensory abilities are not tested, the parent may be deaf or

anomalous due to the mutation, and deficits in parental behaviors can be overinterpreted [9].

4.9. Addictive drugs

Good paradigms are available to assess responsivity to drugs of abuse in mice. Self-administration of drugs of abuse, including alcohol, cocaine, morphine, and nicotine, are measured in mice [1,5,7,13,28,29,43,46,50,61]. Drugs are self-administered intravenously or in the drinking water. Conditioned place preference quantitates the rewarding internal cues created by a drug treatment. Measures of tolerance, dependence, and withdrawal symptoms are quantitated by an observer, using standardized scoring methods.

5. Conclusions

In the opinion of this author, the present technology for the generation of mutant mice is most useful for modeling the symptoms of single gene mutation human hereditary diseases. Behavioral phenotypes identified for transgenic and knockout mice may lead to important medical applications. When the mutation is designed to mimic the genetic mutation in a human hereditary disease, the mouse behavioral phenotype can serve to evaluate the efficacy of new pharmacological and gene therapy treatments [4,6,11,56]. Behavioral phenotypes revealed in the mutant mouse model can thus serve as quantitative surrogate markers to test the efficacy of potential therapeutics.

Two major problems of the current transgenic and knockout technology limit its usefulness to the more general study of the role of genes in normal and abnormal behaviors. The first is the presence of the mutation in all cells. A gene expressed in the brain may also be expressed in many peripheral organs. It is impossible to assign a behavioral difference in the mutant mouse to a specific brain structure or pathway, or even to the nervous system. Tissue specific 'conditional' knockouts are being generated to solve this problem ([35,36,47,73], and see other reviews in this volume). The second is the presence of the mutation from the earliest stages of development. Other genes may compensate during development for the absence of the deleted gene or for the overexpression of the transgene. Lack of behavioral phenotype may be due to compensation by a redundant gene which takes over the function of the missing gene. An opposite behavioral phenotype to that predicted may be due to overcompensation by one or more redundant genes. An observed behavioral phenotype may in fact characterize the role of the compensatory gene, not the targeted mutation. Temporally-selective 'inducible' transgenics are being generated to solve this problem ([36,47], and see other reviews in this volume).

If conditional and inducible mutations become feasible, it is likely that the transgenic and knockout technology

will become the premiere research tool to further our understanding of the genetic basis of behavior. Gene mutations may replace pharmacological tools, such as drugs that act as selective antagonists at neurotransmitter receptor subtypes. Gene mutations may also replace lesions of brain structures or pathways to study the functions of endogenous neurochemicals and neuroanatomical pathways. Behavioral phenotyping methods continue to evolve to optimize our approaches to the growing opportunities of the mutant mouse technology.

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