Chapter 2

Animal Behavior

After reading this chapter, you should be able to:

- Compare and contrast the advantages and disadvantages of using common model organisms to study animal behavior
- Discuss considerations for designing a behavioral assay
- Describe common behavioral assays used in rodents, invertebrates, and primates

Techniques covered:

- **Rodent motor assays**: running wheel, homecage activity, open field test
- **Rodent motor coordination assays**: rotarod test, footprint pattern assay, hanging wire assay, vertical pole test
- **Rodent sensory assays**: visual cliff assay, startle response, taste assays, olfactory assays
- **Rodent nociception assays**: tail flick assay, Hargreaves assay, hot plate assay, Von Frey assay, formalin assay
- **Rodent spatial learning and memory assays**: Morris water maze, Barnes maze, radial arm maze, virtual reality maze
- **Rodent nonspatial learning and memory assays**: classical conditioning, operant conditioning, novel object recognition, delayed match to sample and nonmatch to sample assays
- **Attention and impulsivity**: go/no-go task, five choice serial reaction time task
- **Rodent reward-related assays**: self-administration, progressive ratio, conditioned place preference/aversion, real-time place preference
- **Rodent social assays**: resident−intruder assay, social approach/avoidance assay
- **Rodent anxiety assays**: open field test, elevated plus maze, defensive marble burying, Geller−Seifter conflict test
- **Rodent depression assays**: forced swim test (Porsolt test), tail suspension assay, sucrose preference test
- ***Drosophila* behavioral assays**: locomotion, flight, sensory assays (vision, olfaction, taste), learning and memory assays, courtship, aggression
- ***C. elegans* behavioral assays**: locomotion, sensory assays (mechanosensation, thermosensation, chemosensation)
- **Nonhuman primate behavioral paradigms**

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Humans are the only animals capable of verbally reporting what they feel, what they perceive, and what they know. To gain insight into the emotional, perceptual, and cognitive processes of other animals, a scientist can only observe and analyze their behavior. Over the past several decades, scientists have developed and standardized a variety of behavioral tests to assess the mental state of common model organisms, from their ability to perceive a stimulus, to whether or not they are emotionally depressed. There are now dozens of behavioral assays commonly used in neuroscience studies, with novel assays continually developed to model human behaviors and the symptoms of psychiatric diseases.

The goal of the behavioral neuroscientist is not only to characterize an animal's behavior, but also to identify and describe the genetic, biochemical, and cellular correlates of that behavior. Therefore, behavioral neuroscience does not exist in isolation, but rather in combination with systems and molecular techniques. To correlate electrical events in the brain with specific behavioral outputs, a scientist can monitor neural activity during a behavioral task using electrophysiology or optical methods (Chapters 4 and 7). A scientist can also determine which genes, proteins, and/or neurons are necessary or sufficient for a behavior to occur by investigating the effects of their perturbation (Chapters 4, 8, and 12). Thus, the behavioral paradigms described in this chapter are used in conjunction with many other techniques described in this book.

The goal of this chapter is to describe the general strategies scientists use to measure behavior in rodents, invertebrates, and primates. First, we will discuss some of the issues a scientist must consider when choosing and performing behavioral assays. Then we will survey the behavioral assays that are the most commonly used in the literature.

CONSIDERATIONS FOR CHOOSING AND PERFORMING A BEHAVIORAL ASSAY

There are many factors involved in the design of a behavioral assay. These factors include choosing a model organism, choosing a behavioral paradigm, reducing variability between individuals, and validating animal behavior as a useful model for human behavior and disease.

Choosing an Appropriate Model Organism

A scientist should consider two major factors when choosing an animal model to study behavior: the natural ethological capabilities of an animal, and the utility of the animal for other experiments.

Ethology refers to the natural capability of an animal to perform specific behaviors. Worms and flies can be good models for studying behaviors in

which animals interact with the environment, such as sensory transduction, food seeking, and motor control. Flies have also been used to study addiction, learning and memory, courtship, circadian rhythms, and other behaviors. Mice and rats are able to perform these behaviors and also more complicated cognitive tasks that underlie emotion, cognition, and social behavior. Primates are capable of highly intelligent behaviors, such as complex decision-making, identification of faces, and use of tools. In addition to these standard model organisms, scientists can choose to study other animals with specialized adaptations that make them an optimal model for certain behavioral tasks (Box 2.1).

A scientist must also consider the model organism in the context of other experiments. If the goal of the study is to identify novel genes that regulate a behavior, the best choice of animal model will be a genetically tractable organism, such as a worm, fly, or mouse. If the goal is to record from several neurons in the brain while the animal performs a behavior, a larger animal may be necessary, such as a rodent or primate. Mice are often used in behavioral assays because they are amenable to genetic, molecular, electrophysiological, and imaging approaches.

It is also important to consider the ethical and moral implications of using a specific animal model in an experiment. The use of animals in research is both a necessity and a privilege that a scientist should never fail to appreciate (Box 2.2).

BOX 2.1 Neuroethology

Neuroethology is the study of the neural basis of an animal's natural behaviors. This term can be applied to traditional research animals, like rodents and primates. However, it is more commonly used to describe nontraditional research animals that have evolved unique adaptations to their ecological niches, making them particularly good model organisms for specific research goals. For example:

- Echolocating bats have enlarged brainstem structures useful for studying the auditory system.
- Various species of birds learn and practice song, allowing scientists to study developmental learning, consolidation of learning, and language.
- Barn owls hunt in the dark and have an amazing capacity for sound localization, as well as coordinating visual and auditory information.
- Some species of prairie voles exhibit monogamous behavior and an increased tendency, compared with other rodents, to stay in stable male/female pairs.

Studying these animal models results in not only a greater understanding of the neurobiological basis of ethologically relevant behaviors, but also a greater understanding of human biology. For example, much of our understanding about the human auditory system has come from research on echolocating bats.

BOX 2.2 Ethical Considerations for Animal Use

Using animal models in biological research is necessary to make conclusions about a fully functional, living organism. However, using animals is also a privilege, and scientists deciding to use animal models must consider many ethical considerations. These ethical concerns are not solely the responsibility of the individual scientist; the use of animals must also be justified to animal oversight committees that exist to maintain the welfare of research animals. Each institution has an **Institutional Animal Care and Use Committee (IACUC)** that must approve protocols involving animals and ensure that the research is justified. Furthermore, granting agencies and many scientific journals now require that scientists state that all animal protocols are approved by their institution's IACUC.

When using animals in experiments, distress must be minimized and the potential benefits to humans must be maximized. Animal welfare guidelines throughout the world are based on the principles proposed by Russell and Burch in *The Principles of Humane Experimental Research* (1959), commonly referred to as the three Rs:

Replacement: If there is any way to do the research without using animals, it should be done. This includes using computational modeling and cell culture methods. In the case of behavioral research, modeling is obviously impossible without actually performing the behavioral task. However, another component of replacement is the use of "less-sentient" animal species: choosing to study invertebrates rather than vertebrates, or mice rather than monkeys, etc.

Reduction: Use the minimum number of animals to obtain scientifically valid data. If possible, use the same animals for multiple experiments. Also, investigators should perform mathematical analyses to determine the minimum number of animals required to provide enough statistical power to obtain significant results.

Refinement: Procedures must minimize distress and pain and enhance animal well-being. This guideline is also important for the consistency and interpretability of experimental results. For example, stress has numerous effects on physiology that can confound results.

Following these guidelines is standard practice for developing animal protocols and justifying research goals.

Choosing an Appropriate Behavioral Paradigm

Whether investigating a commonly studied behavior or developing a new behavioral paradigm from scratch, a scientist should consider many factors when preparing to use an assay. First and foremost, the assay must be quantifiable, with the ability to measure discrete, easily observable variables. For example, consider an experiment in which a scientist wishes to measure drinking behavior between two groups of rodents. The scientist has many potential variables to measure: the total volume of liquid consumed, the rate of

water consumption, the number of licks applied to a liquid delivery system, etc. In the literature, data are usually presented as bar graphs or scatter plots, with different graphs representing different quantifiable aspects of each behavioral assay.

A scientist must also consider that animals, especially animals in which an investigator has manipulated gene or protein expression or neural activity, may exhibit abnormal behaviors for reasons the scientist may not expect. For example, animals injected with a pharmacological agent may take a statistically longer time to complete a maze than control animals injected with saline. However, this effect may not be due to problems in spatial navigation or memory, but altered motor behavior that indirectly increases the amount of time spent in the maze. Therefore, when choosing a behavioral assay, scientists must consider alternate explanations for why experimental groups may differ, adding additional behavioral experiments as necessary. It is also important to examine the overall health of the animal (body weight, heart rate, etc.) to ensure that animals do not perform abnormally because they are sick.

Variability in Individuals

There are many factors that can increase the variability of a model organism's performance on a behavioral task, making it difficult to achieve consistent results. One factor is an animal's genetic background, which can have profound effects on behavior. Consider mice as an example: there are over 10 strains of mice commonly studied in research laboratories. Some strains behave very differently than others. For example, C57/BL6 mice are known to be much more aggressive than the FVB strain. An investigator performing social experiments on C57/BL6 mice might therefore observe different results than another investigator performing the same experiments using FVB mice. Strain-specific differences are also present in other model organisms, such as worms, flies, rats, and even primates. Therefore, it is important to always perform behavioral assays in the same strain and, if possible, the same generation of animal. Comparisons between genetically modified animals and wild-type animals should be performed on individuals from the same litter.

Environmental variables can also impact behavior. These variables include animal handling, diet, circadian rhythms, seasonal changes, social interactions, etc. For example, an investigator may observe different behaviors on a Monday compared to a Thursday if it turns out that an animal technician changes the animal cages (and thus handles the animals) each Monday. Alternatively, an individual animal may perform differently on a behavioral assay if it is housed with other animals compared to if it is housed alone. These factors require a scientist to foresee and minimize any aspects of an animal's environment that may introduce variability in behavior. Additionally, scientists may need to perform behavioral assays on large numbers of animals to produce reliable measurements and results.

Historically, scientists have avoided using female rodents for fear of increased variability due to fluctuating hormone levels. This sex bias poses a serious problem, however, when interpreting results. Behavioral studies performed only on male mice cannot be generalized to female mice. Because behavioral studies in rodents may eventually lead to the development of therapeutics for use in humans, it is critical that any sex differences in safety and efficacy are uncovered during preclinical studies. Therefore, it is strongly recommended that scientists use both female and male subjects in their behavioral studies. These studies should have large enough sample sizes to compare male and female rodents, and observed differences should be noted.

Using Animal Behavior as a Model for Human Behavior

One of the primary goals of biomedical research is to learn more about humans by studying animal models. Therefore, it is worthwhile to ask—can scientists extrapolate and generalize the results of an animal behavioral assay to people? Is it possible to model human neurological and psychiatric disorders, such as Alzheimer's disease and depression, using rodents?

The answers to these questions depend on the exact behavior and disease studied, but scientists generally gauge the ability of an animal model to serve as a proxy for a human behavior or disease by examining the model's **validity**. There are three general categories of validity: face validity, construct validity, and predictive validity. A model has **face validity** if the model's behavior/phenotype is similar to the analogous human behavior/phenotype. For example, mouse models of autism spend statistically less time engaging in social behaviors than wild-type mice, just as many humans diagnosed with autism have difficulty interacting socially with others. **Construct validity** refers to a state in which the animal model and human model have the same underlying genetic or cellular mechanism that may result in a certain behavior, such as a transgenic mouse engineered to have the same mutation that causes a disease in humans. A model has **predictive validity** if treatments used in human patients have the same effect on the animal model. For example, some antidepressants prescribed to humans decrease behavioral measures of depression in rodent models.

It is possible for animal models to exhibit one form of validity but not others. The more forms of validity that an animal model exhibits, the greater its relevance and value to humans. Note that validity is not something that can be objectively measured, but is instead a concept judged by scientists and their colleagues within various subfields of neuroscience.

Now that we have discussed some general features of behavioral paradigms, we describe many commonly used assays in modern neuroscience research.

RODENT BEHAVIORAL PARADIGMS

Rodents (especially mice) are perhaps the most commonly used mammalian model organisms in behavioral neuroscience because they can be simultaneously used for molecular, genetic, electrophysiology, and imaging experiments. The following assays represent common approaches used by investigators to measure motor function, sensation, learning and memory, reward-related behaviors, social interactions, and emotion.

Locomotor Activity

Locomotion assays are used to determine the net motor activity of an animal over a given time period. These assays are useful for determining if two cohorts of animals (e.g., knockout animals compared to wild-type littermates) have the same baseline activity. If measurements are recorded continuously over days and weeks, these assays are also useful for studying **circadian rhythms**—the regular, roughly 24-hour-cycle of stereotyped biochemical, physiological, and behavioral activity.

Running Wheel

A running wheel is the simplest way to measure the locomotor activity of an animal over time. The wheel is coupled to a device that measures the total number of revolutions and the speed of each revolution. If the animal is free to enter or exit the running wheel voluntarily (as in most running wheel assays), then the assay cannot measure locomotor activity when the animal is not on the wheel. However, a running wheel is more metabolically demanding than the simple movements that a rodent typically performs around its cage, and therefore running wheels can serve as reliable measurements of activity.

Homecage Activity

Locomotor activity can be measured in specially designed cages that project infrared beams from one side of the cage to the other. Each time an animal moves around the cage, it breaks the beam and a computer records the time and position (Fig. 2.1). Alternatively, a scientist can place a camera above the cage and record activity. Computer tracking programs can be used to statistically analyze the total locomotor activity over time. Depending on the specific setup, a scientist can examine horizontal activity, vertical activity, time spent in various regions of the cage, and total distance traveled.

Open Field Test

An **open field test** utilizes a large cubic box (Fig. 2.2). The top of the cube is typically left uncovered. An animal is placed in the middle of the bottom surface, and its movements are recorded over the course of minutes to hours as

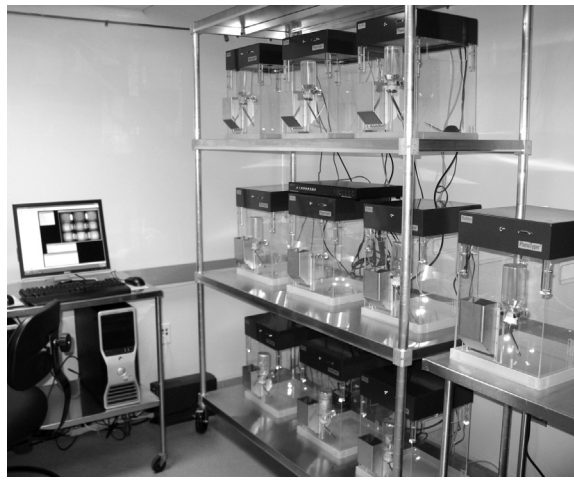

FIGURE 2.1 Locomotor activity can be monitored in specially designed home cages. *Courtesy of the Stanford Behavioral and Functional Neuroscience Laboratory.*

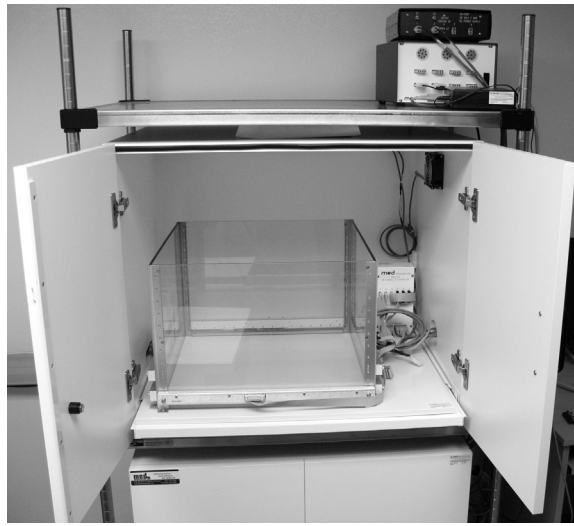

FIGURE 2.2 Exploratory locomotor activity can be monitored in an open field testing chamber. *Courtesy of the Stanford Behavioral and Functional Neuroscience Laboratory.*

it moves around and explores its environment. After the experiment is completed, computer tracking programs analyze the movements of the animal over time. This assay can measure horizontal activity, time spent in various regions of the open field, and the total distance traveled. This assay can also be used to measure anxiety (described later in assays of stress).

Motor Coordination and Balance

Assays for motor coordination and balance are used to test the strength of an animal's motor and vestibular systems. These tests can be used in isolation or to exclude motor deficits as a reason for poor performance in other behavioral experiments. For example, if pharmacological or genetic manipulation of an animal results in abnormal performance on a learning and memory assay, a scientist could ensure that this abnormal behavior is not due to general motor deficits.

Rotarod

A **rotarod** is a device consisting of a cylindrical rod suspended above a padded base (Fig. 2.3). A scientist places a rodent on the rod and gradually accelerates the rotation until the rodent falls to the bottom of the chamber. Most animals are able to maintain balance for many minutes, but rodents with deficits in motor coordination or balance fall more quickly. This assay can also be used as a measure of motor learning, as most animals perform better on the rotarod assay after consecutive training days.

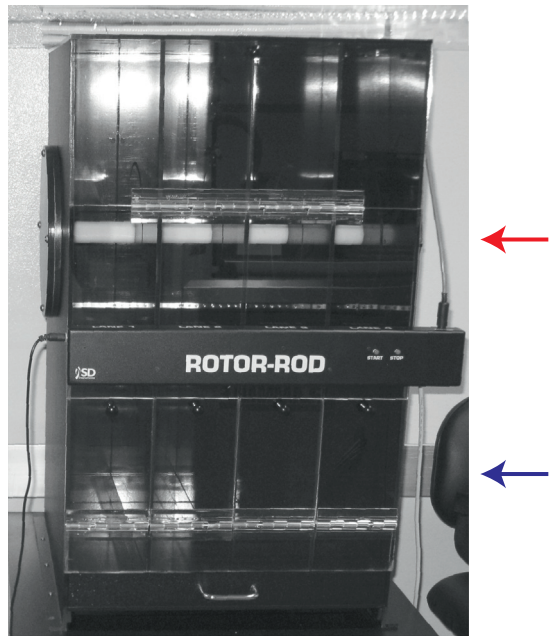

FIGURE 2.3 A rotarod tests balance and motor coordination. Animals are placed on a rotating rod (*red arrow*) until they eventually fall onto a padded floor (*blue arrow*). Multiple animals can be tested at one time with a device that has individual lanes to record each animal's latency to fall. *Courtesy of the Stanford Behavioral and Functional Neuroscience Laboratory.*

Footprint Pattern Assay

A scientist performs a **footprint pattern assay** by dipping an animal's paws in ink and allowing it to walk down a paper-lined tunnel. These footprints can reveal gait abnormalities and **ataxia**—abnormal muscle coordination (Fig. 2.4). Dipping the forepaws and hindpaws in different colors of ink allows a scientist to measure a variety of gait parameters: distance between each stride, changes in stride length, variability around a linear axis, width between left and right hindpaws, regularity of steps, and overlap between fore- and hindpaws. Modern automated versions of this task can also track pressure and walking speed. Ataxic gait is characterized by a highly variable stride length and path.

Hanging Wire Assay

The **hanging wire assay** is used to measure neuromuscular deficits. An investigator places a rodent on a cage lid made of a wire grid, shaking the lid so the rodent grips the wire. The investigator then gently flips the lid so the rodent hangs upside down. To prevent falling, the rodent grasps the wire, requiring both balance and grip strength. The investigator measures the time it takes before the rodent falls to a padded surface. A normal rodent can hang upside down for several minutes, but a 60-second cutoff is often used for experimental purposes.

Vertical Pole Test

In a **vertical pole test**, a rodent is placed on the center of a horizontal pole wrapped in cloth to provide traction. The pole is gradually lifted toward a

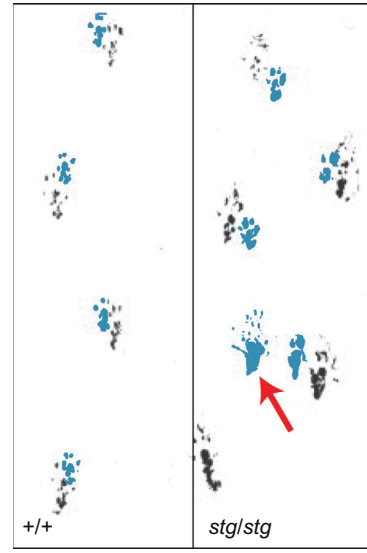

FIGURE 2.4 Footprint pattern analysis can reveal gait abnormalities. Footprint patterns of a wild-type (+/+) mouse compared to a mutant (stg/stg) mouse. The mutant mouse exhibits a wider stance (proximity of blue forelimb and black hindlimb footprints) and stumbling (*red arrow*). *Reprinted from Meng, H., et al., 2007. BDNF transgene improves ataxic and motor behaviors in stargazer mice. Brain Res. 1160, 47–57, with permission from Elsevier.*

vertical position, and the investigator records the amount of time and the angle at which the animal falls off. Normally, rodents can move up and down the pole without falling before the pole reaches a 45-degree angle.

Sensory Function

Abnormal sensory function is usually more difficult to observe than abnormal motor function, as it is often impossible to directly measure what a rodent perceives. In many sensory assays, a rodent uses motor output to report detection of a sensory stimulus; thus, these assays are sensitive to confounding effects due to deficits in motor function.

Visual Cliff Assay

A **visual cliff assay** evaluates the ability of a rodent to see the drop-off at the edge of a horizontal surface. The edge of a box or table can serve as a horizontal plane with a vertical drop to a lower horizontal plane, such as the floor. Each surface is coated with a black-and-white checkerboard pattern to enhance the detection of visual planes (Fig. 2.5). A clear sheet of plexiglass is placed over the drop-off, extending across the "cliff" so an animal cannot actually fall. Normally, rodents visually detect the drop-off and approach the edge cautiously, stopping at the cliff. Blind rodents approach the cliff but continue walking, unable to see the edge but able to feel the plexiglass sheet. Rodents can also use their whiskers to feel the plexiglass extension, so the whiskers are often cut prior to an experiment.

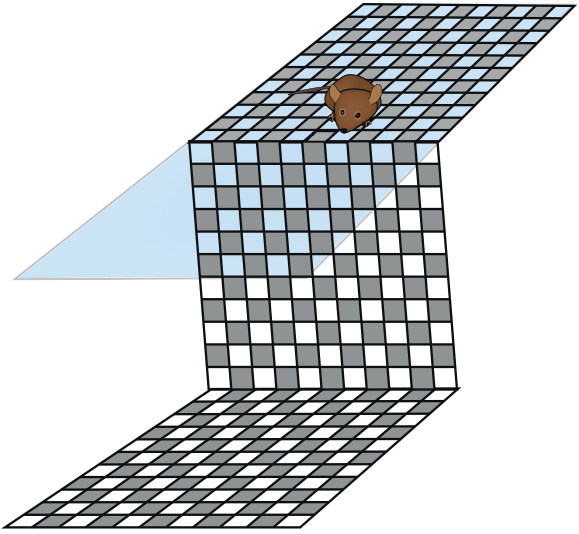

FIGURE 2.5 **Visual cliff assay.** A checkerboard pattern enhances the perception of a drop-off. Rodents that can see this drop-off will pause at the edge, while rodents with visual deficits will feel the plexiglass surface and continue walking without pause.

Startle Response Assay

In a **startle response assay**, a scientist exposes a rodent to an unexpected and disruptive sensory stimulus and measures the degree to which the animal responds, typically exhibited as an eye blink, body flinch, or overall muscle contraction. These stimuli can be visual (a bright light), auditory (loud and disrupting noise), or tactile (unexpected touch or air puff). Investigators can either observe and score the number of times a startle response occurs or use specialized sensors, such as an **electromyogram**, to more quantitatively measure the degree of the response. This assay can be confounded by deficits in emotional and motor components of the startle reflex circuitry. This assay can also be used to measure animal learning by measuring extinction of the startle response over successive trials.

Interestingly, a startle response can be reduced if a nonthreatening stimulus is presented immediately before the disruptive sensory stimulus. This phenomenon is known as **prepulse inhibition (PPI)**, and reflects the nervous system's ability to prepare for a strong sensory stimulus after a small warning (the prepulse). Human patients with attention deficit disorder, Alzheimer's disease, and schizophrenia show deficits in PPI. Therefore, PPI can be a useful paradigm to test animal models of psychiatric disease and establish face validity for animal experiments. Furthermore, PPI has been a useful behavioral assay to test potential antipsychotic medications that improve sensory processing.

Taste/Flavor Assays

Investigating an animal's ability to perceive taste or flavor usually involves a choice assay, in which different gustatory compounds are placed in different solutions in water bottles. The scientist measures the volume of water in each bottle to determine the animal's taste/flavor preferences. It is possible to investigate whether an animal can detect bitter tastes by placing quinine, an extremely bitter compound, in a water solution and determining if the animal consumes less of the quinine solution than a solution with no quinine. Likewise, it is possible to investigate whether an animal can detect sweet tastes by placing sucrose or saccharine in a solution and determining if an animal drinks more of the sweet solution than a control solution with no sweet compounds.

Olfaction Assays

A scientist can test for normal olfactory processing by measuring how long it takes for an animal to find a piece of familiar, palatable food (such as a cookie, chocolate chip, or cheese) hidden within the cage. Olfaction can also be used to test fear responses by placing a cotton ball with TMT, a component of fox feces, in the cage. Mice innately fear the smell of TMT and will avoid the area of the cage where the cotton ball is placed.

Nociception

Nociception is the ability to detect a noxious stimulus, usually perceived as pain. Nociceptive assays rely on physical indicators of discomfort, such as withdrawal reflexes, licking, and vocalizations. These assays are usually used to investigate either the neural basis of pain or the therapeutic potential of analgesic drugs. Genetically or pharmacologically manipulated animals that exhibit decreased sensitivity to pain must be monitored closely during these assays to prevent tissue damage induced by nociceptive stimuli.

Tail Flick Assay

In the **tail flick assay**, a high-intensity beam of light is aimed at a rodent's tail. Alternatively, the tail is placed in hot or cold water. In normal animals, these stimuli produce a painful sensation, causing a reflex that moves the tail. The investigator measures the amount of time it takes before the animal flicks its tail to the side. Sex, age, and body weight can affect a rodent's response, so all animals used for these experiments (experimental and control animals) must be similar to avoid confounding results.

Hargreaves Assay

The **Hargreaves assay** is similar to the tail flick assay, but the high-intensity beam of light is aimed at the rodent's hindpaw rather than the tail. The investigator measures the time it takes for the rodent to withdraw its paw.

Hot Plate Assay

In the **hot plate assay**, a scientist places a rodent on a heated surface and prevents the animal from leaving by enclosing it within a tall cylinder (Fig. 2.6). The surface is calibrated so that a normal animal will react within about 10 s of exposure (usually 52−53°C). The investigator records the latency and amount of time an animal reacts to the heat stimuli. Individual reactions vary: licking a rear paw is a reliable indicator of discomfort, and some animals will jump or vocalize.

Von Frey Assay

A **Von Frey assay** is used to examine sensitivity to pinch and mechanical stimuli. Von Frey hairs are fine-gauge metal wires. The investigator pokes the hindpaw of an animal standing on an elevated mesh platform by inserting a Von Frey hair through the mesh from below. Normal rodents usually react by withdrawing or licking their paws and possibly vocalizing.

Formalin Assay

A **formalin assay** is used to examine an animal's sensitivity to noxious chemical stimuli. An investigator injects a small volume of formalin, a noxious

FIGURE 2.6 Hot plate test of pain perception. *Courtesy of the Stanford Behavioral and Functional Neuroscience Laboratory.*

chemical, into a rodent's hindpaw and records the total amount of time the rodent spends licking, biting, or withdrawing the affected limb. This activity is usually assessed in two phases: one phase beginning immediately after the injection and lasting about 10 min and another phase starting 20 min after the injection and lasting about an hour. The second phase is a response to tissue damage caused by inflammation.

Spatial Learning and Memory

In the wild, rodents spend most of their lives locating good nesting spots, searching for food, and avoiding predation. Therefore, rodents are ethologically relevant model organisms for assays that test spatial learning and memory.

Morris Water Maze

The **Morris water maze** is one of the most frequently used spatial learning and memory paradigms. Rodents are placed in a large circular pool of opaque water (Fig. 2.7). Their natural dislike of water makes them highly motivated to escape from the pool. In initial trials, a visible platform is placed within the pool so that an animal can emerge from the water. A scientist places visual cues around the pool so the animal can relate the spatial environment with the location of the platform. In subsequent trials, the platform is hidden just below the surface of the water, and a scientist measures the time required for the rodent to swim to the platform to escape the water.

FIGURE 2.7 **The Morris water maze.** Animals are placed in circular bath of opaque water and learn to find a slightly submerged platform. The patterns on the wall surrounding the bath provide visual cues to help the animals locate the platform on subsequent trials. *Courtesy of the Stanford Behavioral and Functional Neuroscience Laboratory.*

In the first few trials, the rodents become progressively better at swimming directly toward the platform, demonstrating their ability to learn the platform's location. To test memory at the end of training, the investigator completely removes the platform to measure the amount of time the animal spends searching in each quadrant of the pool. This experiment measures the ability of the animal to identify a spatial location based on the visual cues placed around the chamber. Because water is stressful to rodents (in mice more than rats), it is important for a scientist to account for stress-induced performance effects.

Barnes Maze

The **Barnes maze** consists of a circular table with holes around the circumference, placed in a room with visual cues in the periphery (Fig. 2.8). Most of these holes lead to an open drop to the floor, but a single hole leads to a "drop box," a dark box in which the animal can hide. A rodent is naturally motivated to avoid open spaces and bright lights, and therefore attempts to find the drop box. In initial trials, the scientist gently leads the animal to the drop box. In subsequent trials, the animal is placed in the center of the table and must find the drop box on its own. After a few trials, rodents typically remember which hole contains the drop box and quickly proceed in a direct path toward the hole. Investigators can measure the amount of time to find the correct hole, the number of incorrect holes explored, and the length of the exploratory path. The Barnes maze is less stressful to rodents than the Morris water maze.

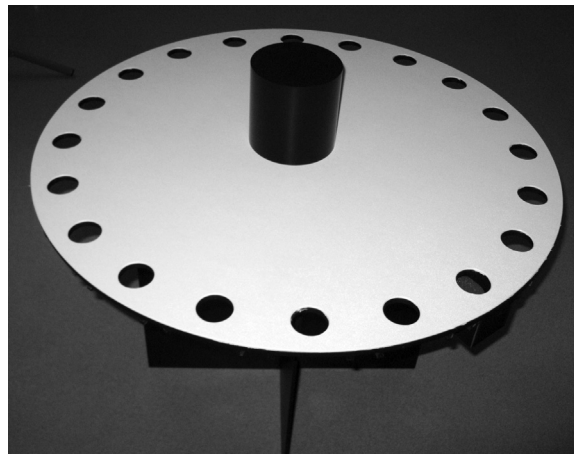

FIGURE 2.8 **The Barnes maze.** Rodents learn which one of the holes contains a hidden drop box they can use to escape from light. *Courtesy of the Stanford Behavioral and Functional Neuroscience Laboratory.*

Radial Arm Maze

The **radial arm maze** consists of an array of "arms," usually eight or more, that radiate from a central starting point (Fig. 2.9). At the end of each arm is a cup that may or may not contain a food reward. Animals are trained to

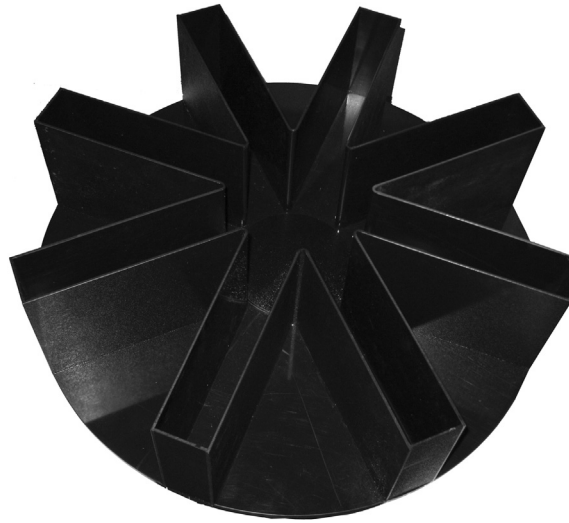

FIGURE 2.9 **Radial arm maze.** *Courtesy of the Stanford Behavioral and Functional Neuroscience Laboratory.*

recognize that only one of the arms will contain food. Investigators measure the amount of time it takes for an animal to find the arm leading to food, as well as the number of times it traverses an arm it has previously visited. Exploring a previously visited arm indicates that the animal did not remember previously choosing that spatial path. This task is relatively difficult for rodents, requiring several days or weeks to train rats and many weeks to train mice. Unlike the Morris water maze and Barnes maze, the radial arm maze does not use distant visual cues to aid spatial learning.

Virtual Reality Mazes

Spatial learning and memory tasks can also be performed using virtual reality. Typically, a mouse's head is fixed into position and is allowed to run on a foam ball that spins underneath, like a treadmill. A screen projects a virtual scene, like a maze, in front of the mouse. The rotation of the foam ball is measured and used to advance the position of the mouse in the maze, allowing it to search for a virtual object to obtain a reward. This reward is typically a sweet solution delivered through a spout near the mouth. The ability of the mouse to navigate an environment while head restrained allows for simultaneous monitoring of neural activity using electrophysiology or calcium imaging.

Nonspatial Learning and Memory

The neural circuitry that mediates nonspatial learning and memory differs from circuitry that mediates spatial learning and memory. Thus, multiple assays using both nonspatial and spatial-based tasks are used to evaluate learning and memory as a whole. Nonspatial learning and memory assays include innate learning, such as motor performance improvement over time (such as the rotarod assay), novel object recognition, as well as learned associations through conditioning training. While classical (Box 2.3) and operant (Box 2.4) conditioning can be used to directly assay an animal's ability to learn, these paradigms are often used to train and test animals on more sophisticated tasks. For example, the delayed match to sample and nonmatch to sample assays use operant conditioning techniques to examine an animal's working memory. These techniques are described below.

Novel Object Recognition

The **novel object recognition** paradigm assesses an animal's innate ability to distinguish an old object from a new object. Rodents are naturally curious and will spend time exploring novel objects. When presented with both a new object and a previously explored object, normal rodents remember the old object and spend relatively more time exploring the new object. A scientist measures the amount of time spent interacting with both objects.

BOX 2.3 Classical Conditioning

In **classical conditioning** (also called **Pavlovian conditioning**), the investigator couples an initially neutral stimulus, such as a tone or light, with a salient stimulus, such as food or an electric shock. The neutral stimulus is referred to as the **conditioned stimulus (CS)**, while the salient stimulus is referred to as the **unconditioned stimulus (US)**. The unconditioned stimulus elicits a reflexive response, such as salivation or fear behaviors. By pairing the conditioned and unconditioned stimuli, the conditioned stimulus alone is eventually able to elicit the reflexive response of the unconditioned stimulus. Examples of Pavlovian conditioning are **cued fear conditioning** and **contextual fear conditioning**. Fear conditioning often utilizes a foot-shock (US) to induce freezing behavior. By associating an auditory or visual cue (CS) with the foot-shock, animals will freeze in response to the sensory cue alone (i.e., without any foot-shock) demonstrating a learned association. Contextual fear conditioning measures freezing behavior when the animal is returned to the training chamber without foot-shock—exhibiting a response without the unconditioned stimulus shows that the animal has learned the association between a location and the shock. Cued conditioning should be measured in a new testing chamber with a very different context (shape of the box, visual cues, lighting, odors, textures), but with the same auditory or visual cue from training to test the specific learned association between the cue and the shock.

BOX 2.4 Operant Conditioning

In **operant conditioning**, a voluntary response (e.g., a lever press or a nose poke) is induced by positive reinforcement or suppressed by punishment. Positive rewards often include food or water (especially in food- or water-deprived animals) or drugs of abuse. Punishing or aversive stimuli often include foot-shocks or an unpleasant sensory stimulus such as a loud tone. Several training sessions are often required for animals to learn the association between performing a task and receiving reinforcement or punishment. While operant conditioning is a form of learning, this paradigm is often used to study other behaviors, such as reward, attention, and impulsivity.

Delayed Match to Sample/Nonmatch to Sample Task

Operant conditioning (Box 2.4) can be used to train an animal to make a response such as poking its nose into a hole or pressing a lever for a reward in response to a stimulus, often a light appearing above the lever. To test working memory, the investigator trains the rodent to perform an operant response under specific conditions—for example, pressing a specific lever when multiple levers are present. In a **match to sample task**, a light appears above one lever, and the animal must choose to press the lever beneath that light. In a

nonmatch to sample task, a light appears, and the animal must choose to press the opposite lever. After learning these tasks, the scientist adds artificial delays, such as withdrawing the lever, between the time when a cue appears and the time that an animal responds. The longer the delay, the longer the animal must remember the light cue that was presented, thus testing the animal's working memory.

Attention and Impulsivity

Rodent models of attention and impulsivity test an animal's ability to learn a relatively difficult task in which success is dependent on sustained attention to a cue and reduced desire to impulsively engage in behavioral responses. These tasks are performed using rats more often than mice because mice often have difficulty learning the task paradigms.

Go/No-go Task

A **go/no-go task** is a form of operant conditioning (Box 2.4) in which a rodent is trained to produce a response, such as a lever-press, in the presence of a specific "go" cue (such as a green light), yet withhold a response in the presence of a separate "no-go" cue (such as a red light). To encourage responses to the "go" cue and to deter responses to the "no go" cue, the "go" cue is often paired with a reward, such as a sweet food, and the "no go" cue is often paired with a punishment, such as a mild shock to the tail or delivery of a bitter taste. Rodents are relatively successful on the "go" trials but often have difficulty withholding responses on the "no-go" trials. These success rates are exacerbated in animal models of impulsivity or low attention span. The motivational state of an animal can also influence success rates—for example, a hungry mouse will respond correctly to a "go" cue paired with food more often than a sated mouse.

Five Choice Serial Reaction Time Task

In the **five choice serial reaction time task**, an animal is placed in an operant chamber (Box 2.4) with five lights coupled with five trays capable of delivering a reward, such as a food pellet. At the beginning of a trial, one of the lights is briefly illuminated, and the rodent must indicate via nose poke or lever-press which light was on. Controlling the length of time the light is on prior to requiring the animal to produce an action can vary the difficulty of the task. Furthermore, this task features an intertrial interval during which an animal must withhold all responses, demonstrating inhibitory control and reduced impulsivity.

Reward-Related Behaviors

Scientists can measure the rewarding aspects of a stimulus, such as an addictive drug or food, by measuring how much an animal is willing to work to

obtain the reward or the degree to which the animal associates the stimulus as a positive reinforcement. These assays are frequently used to study the neurobiology of addiction as well as motivational behaviors such as hunger and thirst.

Self-Administration

Self-administration is a form of operant conditioning (Box 2.4) in which animals are trained to perform a particular action, such as pressing a lever, to receive a rewarding stimulus, such as an addictive drug. Animals are allowed to perform the action freely and repetitively, demonstrating the intrinsically rewarding properties of the stimulus. In a **progressive ratio** experiment, the animal must perform an action progressively more times to receive the rewarding stimulus (for example, pressing a lever 1 time, then 2 times, then 4 times, then 8 times, etc.), and the scientist measures the breakpoint at which the animal is no longer willing to work to receive the reward. Therefore, the progressive ratio task measures an animal's motivation to work for a reward.

Conditioned Place Preference/Avoidance Assay

The **conditioned place preference/avoidance** assay is a form of classical conditioning (Box 2.3) in which a rodent associates a particular environment with a rewarding experience. An animal is placed inside a test chamber (the conditioned stimulus) and allowed access to an unconditioned stimulus (a drug, food, odor, activation of a particular brain region, etc.) for minutes each day for multiple days. The animal is also placed in a control chamber without any stimulus present for minutes each day for multiple days. The two chambers typically differ in color, pattern, and floor texture, so that the animals can associate these cues with the unconditioned stimulus. On the test day, the animal is allowed free access to both chambers, and the scientist measures the amount of time the animal spends in one chamber relative to the other. If animals spend significantly more time in the chamber with the unconditioned stimulus, they are said to have developed a conditioned place preference, associating that chamber with the reward. Alternatively, the unconditioned stimulus could be relatively unpleasant for the animals, and could lead to conditioned place avoidance.

Real-Time Place Preference Assay

The **real-time place preference** assay is similar to the conditioned place preference assay, except that scientists measure the inherent reward value of a certain state without conditioning. Typically, a specific population of neurons is activated or silenced using a neuromodulation method with high temporal specificity, such as optogenetics (Chapter 8), but only when the animal enters one side of the chamber. Mice quickly learn to stay in the side of the chamber associated with the manipulation if it is inherently pleasant, or avoid that side of the chamber if it is unpleasant.

Social Behaviors

Rodents display a variety of innate social behaviors. Mating, parenting, nesting, and grooming are all innate behaviors that can be measured within an individual's cage. Rodents also exhibit specific behaviors based on social recognition when multiple animals are exposed to one another. Exploratory sniffing allows animals to decide on an appropriate social response, such as defending territory or attempting copulation. Two standard assays are well represented in the literature: the resident–intruder assay and social approach/avoidance assays.

Resident–Intruder Assay

The **resident–intruder assay** measures territorial behavior in males. A scientist adds an "intruder" animal to the cage of a "resident" animal and measures specific aggressive behaviors: tail rattling, quivering or thrashing of the tail, physical attacks such as biting or clawing, wrestling, and chasing. The investigator can then compare the amount of time each animal spends investigating the other (following, sniffing, grooming) compared to the amount of time displaying aggressive behavior.

Social Approach/Avoidance

If a scientist presents a rodent with a choice between interacting with another (unfamiliar) rodent or interacting with an inanimate object, most animals will spend more time interacting with the other rodent, a phenomenon referred to as **social approach**. Animals with abnormal social behaviors may spend relatively little time with the other animal, referred to as **social avoidance**. For example, in some mouse models of autism, there is no difference in the amount of time spent exploring an object and exploring the other mouse, similar to asocial behaviors exhibited by humans diagnosed with autism.

Anxiety

Paradigms that measure anxiety generally involve placing rodents in naturally stressful environments, such as brightly lit, open spaces. These assays are useful for investigating brain regions that regulate anxiety, as well as testing novel anxiolytic drugs hypothesized to reduce anxiety. In addition to the common assays described here, investigators can measure physiological correlates of anxiety, such as changes in cardiovascular parameters, defecation, or freezing behavior.

Open Field Test

The **open field test** (Fig. 2.2), previously described as an assay of locomotor activity, can also be used to measure anxiety. A rodent placed in a bare, open chamber will initially stay near the walls and avoid the center (Fig. 2.10).

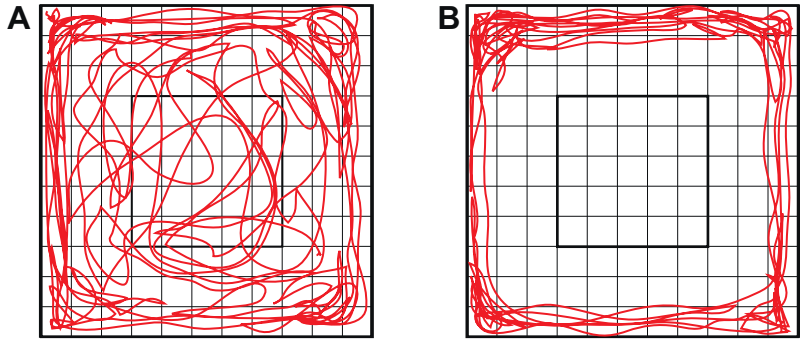

FIGURE 2.10 The open field test as a measure of anxiety. (A) After being acclimated to the chamber, rodents will normally explore the entire area (pathway of movement indicated in red), but (B) a rodent model of anxiety will stay near the perimeter of the chamber.

Normal animals typically acclimate to the chamber and eventually explore the center area. More anxious animals spend significantly less time in the open area and more time closer to the walls. The investigator monitors the animal's activity with a camera placed above the open field, and computer software measures the time spent in the center compared with the periphery. Anxiolytic drugs increase the amount of time rodents spend in the open area.

Elevated Plus Maze

An **elevated plus maze** is a four-armed platform resembling the shape of a plus sign positioned two to three feet above the ground (Fig. 2.11). Two arms of the maze have tall side walls and two have no walls. A scientist places an animal in the center of the maze and allows it to move freely in any of the arms. Animals normally avoid the open arms, spending relatively more time in the protected, closed arms. Anxiolytic drugs increase the proportion of time rodents spend in the exposed arms.

Defensive Marble Burying

Rodents tend to bury objects present in their environment, such as glass marbles, within the bedding of their cages. Animals that are more anxious tend to bury more objects in the 30–60 min after they are introduced. To perform a **defensive marble burying assay**, a scientist places 10–20 marbles in an animal's cage and quantifies the number of marbles buried after 30 min. Anxiolytic drugs tend to decrease the number of marbles buried over time.

Geller-Seifter Conflict Test

The **Geller-Seifter conflict test** is one of the oldest assays to study anxiety and remains a robust test of anxiolytic drug effects. A scientist uses operant conditioning (Box 2.4) to train an animal to press a lever for a food reward.

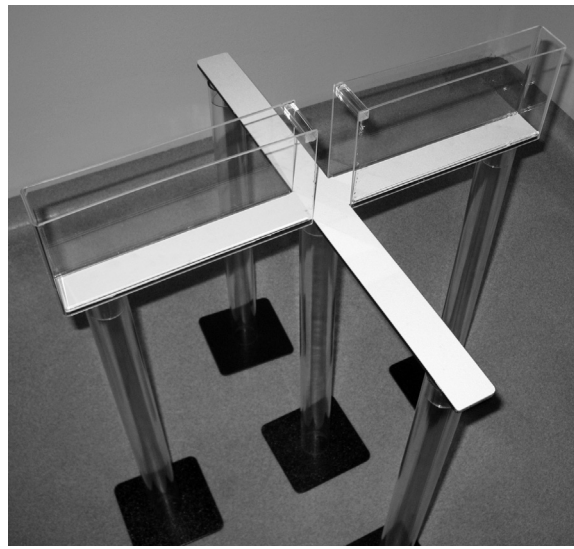

FIGURE 2.11 **The elevated plus maze.** *Courtesy of the Stanford Behavioral and Functional Neuroscience Laboratory.*

During the experiment, the investigator pairs a lever-press with an unpleasant electrical shock. Thus, the animal must decide whether to receive food while getting shocked or not receive food at all. Anxious animals tend to press the lever significantly fewer times than nonanxious animals. Anxiolytic drugs (but not other psychoactive drugs) increase the number of lever presses.

Depression

How is it possible to measure depression in a mouse or rat? Paradigms that investigate depression in animal models assay for the feelings of hopelessness and despair experienced by humans diagnosed with depression. Scientists can induce depressive-like behaviors in a rodent by applying conditions of learned helplessness and chronic mild stress. The **learned helplessness** paradigm exposes an animal to aversive stimuli at random intervals. Theoretically, this treatment creates a condition in which the animal experiences a lack of control, exhibiting symptoms of behavioral despair. Applying conditions of **chronic mild stress** (leaving the lights on all the time, tilting the animal's cage, wetting the animal's bedding, applying intermittent air puffs, etc.) over a period of days to weeks can also theoretically lead to conditions of behavioral despair and helplessness. Both learned helplessness and chronic mild stress cause differences in behavior on the assays of depression described here. Importantly, antidepressants reverse these behaviors, demonstrating the predictive validity of these animal models.

Forced Swim Test (Porsolt Test)

In the **forced swim test** (also called the **Porsolt test**), rodents are placed in a small, confined space, such as a large graduated cylinder filled halfway with water (Fig. 2.12). Initially, there is a period of vigorous activity during which the animal tries to escape. Eventually, the animal ceases vigorous activity and exhibits a characteristic immobility in which it only moves to maintain its head above water. This physical immobility serves as an indication of behavioral despair. Investigators measure the amount of time between when the animal is placed in the chamber and the onset of immobility. Rodent models of depression exhibit a decrease in the time spent trying to escape, and this decrease is reversed with antidepressants.

Tail Suspension Assay

In the **tail suspension assay**, a rodent is suspended in the air by its tail. The animal's natural reaction is to move vigorously to escape. Depressed animals give up more quickly than nondepressed animals. Antidepressants increase the amount of time the animals struggle.

Sucrose Preference Test

The **sucrose preference test** models **anhedonia**—the loss of motivation to pursue an activity that is normally rewarding. Normally, rodents that are allowed to choose between drinking water or a sucrose solution will choose the sucrose solution. Rodent models of depression have a significantly reduced preference for sucrose solution. Importantly, this effect is reversed by administration of antidepressants.

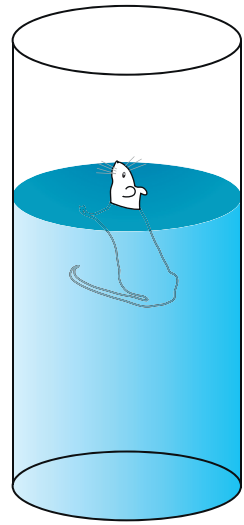

FIGURE 2.12 Performance on the forced swim (Porsolt) test is used as a test of behavioral despair to model depression in rodents.

DROSOPHILA BEHAVIORAL PARADIGMS

One of the powerful advantages of using *Drosophila melanogaster*, the fruit fly, as a model organism is its genetic tractability (see Chapter 9). *Drosophila* has proven useful for studying the genetic basis of behavior because of the fly's relatively short reproduction and developmental period, a large number of genetic tools, and fly-specific behavioral assays. The lab of Seymour Benzer famously used *Drosophila* to study genes necessary for learning, memory, vision, circadian rhythms, nociception, and many other behaviors. Many other labs have used *Drosophila* to study sleep, addiction, courtship, and aggression.

As with any animal model used to study behavior, age, diet, and environmental factors must be strictly controlled when performing behavioral experiments to ensure consistent results. If experiments are performed over multiple days, the scientist should perform assays at the same time each day to avoid potentially confounding results due to circadian rhythms. Handling the flies or administering anesthesia just prior to behavioral tests can also affect the results.

Locomotor Behavior

Locomotor behavior in flies can be studied similarly to locomotor behavior in rodents. A scientist can project infrared beams of light through a housing chamber, allowing flies to break the beams and thus indicate their movement over time. Alternatively, a scientist can record a housing chamber with a video camera, and tracking software can record the activity of many flies over time. Locomotor behavior is useful when studying motor function, circadian rhythms, and the effects of alcohol and other drugs.

Flight

Traditional locomotor assays measure movement in an enclosed container. Another ethologically relevant movement for a fly is flight. Therefore, scientists have developed specialized **flight simulators** that suspend a fly from a thin pin and project visual stimuli to depict the external, visual environment (Fig. 2.13). These simulators contain torque meters to record turning responses to various stimuli, allowing analysis of sensory and motor reflexes. These flight simulators can also be used to study sensorimotor processing and spatial learning and memory.

Sensory Function

Sensory modalities commonly studied in the fly include vision, olfaction, and taste. These modalities are all ethologically relevant for a fly, which spends most of its life navigating through space using visual and chemical cues in search of food or responding to chemical signals produced by other flies.

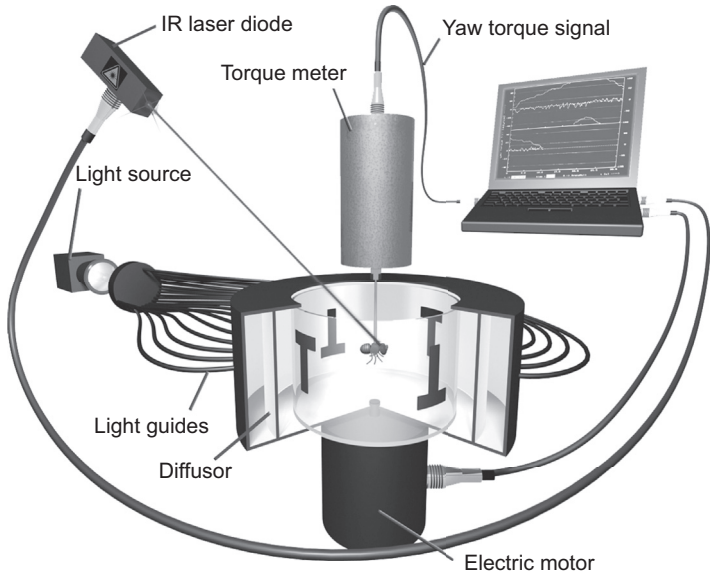

FIGURE 2.13 **A *Drosophila* flight simulator.** This device can be used to measure free-flight behaviors or flight responses to visual stimuli, as well as to train flies by using operant conditioning methods that use a laser to generate heat reinforcement. *Courtesy of Dr. Björn Brembs.*

Vision Assays

Flies exhibit a natural tendency to move toward light, a phenomenon known as **phototaxis**. To test proper visual function, scientists can use a device called a **countercurrent apparatus**, made up of a series of test tubes (Fig. 2.14). Flies are placed in one test tube, which is gently tapped so that the flies fall to the bottom. The test tube is then laid horizontally across from a second test tube. This second tube is illuminated with a fluorescent light. The scientist waits approximately

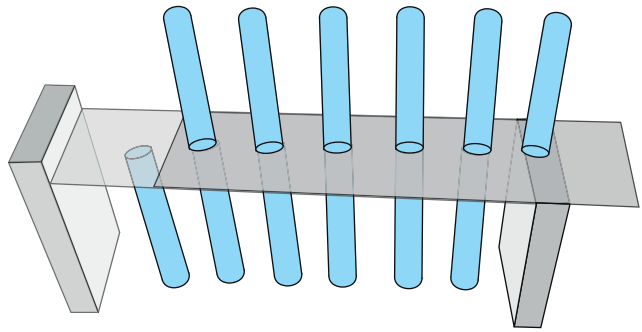

FIGURE 2.14 **The countercurrent apparatus can be used to isolate a population of phototaxing flies.**

15 s before preventing the flies from crossing between the two tubes. The flies trapped within the second tube are considered to have demonstrated more of a phototaxic response. This process is repeated several times, with flies demonstrating a phototaxic response used in each subsequent trial. The end result is a fractionated population of flies that vary in their phototaxic responses.

Vision can also be assayed using a device called a **T-maze** (Fig. 2.15). This apparatus consists of two arms oriented in opposite directions—a "T" shape—with an additional loading arm. Flies are placed into the loading arm and moved to the center of the other two arms of the T-maze, where they can travel to either arm. Each arm contains a different visual stimulus—for example, two different wavelengths of light, bright light versus darkness, flashing light versus constant light, and so forth. Thus, a T-maze allows scientists to understand the visual preferences of a fly, as well as to investigate the genes necessary and/or sufficient for these preferences to occur.

Olfaction Assays

Olfaction assays can also take place in a T-maze. Each arm of the maze contains a different odor, and flies decide which odor to move toward. Investigators can use this assay to determine whether an odor is attractive, repulsive, or neutral.

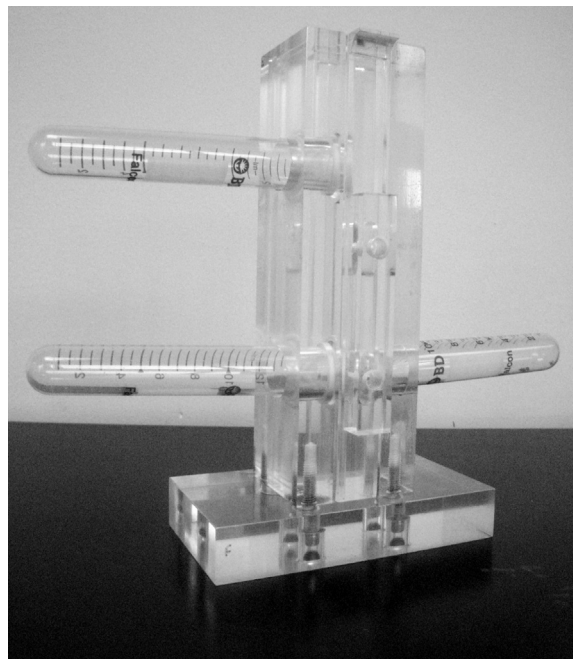

FIGURE 2.15 **A *Drosophila* T-maze can be used to test fly sensory preferences or to train and test flies on conditioning training.**

In an olfactory trap assay, odors absorbed on a cotton swab or filter paper are placed at the bottom of a chamber with a pipette tip that allows flies to enter but not leave. By simply waiting a certain amount of time for flies to experience the baited odor, an attraction index can be calculated from the ratio of flies trapped in the odor vial compared to a water control. An attraction index of 1 indicates complete attraction to the odor, while an attraction index of 0 indicates either no preference for an odor or anosmic flies.

There are many simple avoidance assays that assess behavioral responses to olfactory cues. In the **olfactory avoidance assay** (also called **dipstick assay**), a scientist inserts a piece of filter paper or cotton swab containing a specific odor into a fly vial, then measures the distance that the fly maintains from the offending odorant. A similar assay is the **chemosensory jump assay** or **olfactory jump response**, which exploits the tendency of flies to exhibit a startle response when encountering a novel odor.

Taste Assays

Hungry flies exhibit an unconditioned reflex called the **proboscis extension response**. A scientist can elicit this response by applying taste ligands to gustatory receptor neurons on the leg or nose. This reflex can be used to test specific gustatory receptor responses and sensitivity to different taste ligands.

A scientist can also assay taste using a **feeding acceptance assay**. Starved flies are provided a choice between appetitive, aversive, and neutral stimuli, each dyed a different color. The amount of each stimulus ingested in the dark can be scored by examining the color of the fly's stomach.

Learning and Memory

Learning and memory assays can be performed in *Drosophila* using a T-maze and classical conditioning (Box 2.3). An odor is paired with an electric shock. This odor is then presented in one arm of a T-maze. Flies that avoid this arm are considered to have learned the association. This assay has been widely used in genetic screens that assay both learning and memory, as well as olfaction.

Investigators can also assay learning and memory on individual flies in the flight simulator through classical or operant conditioning. For example, flies can be trained to navigate a virtual scene to find an appetitive sensory stimulus or reward.

Social Behaviors

There are many social behaviors that a scientist can study using *Drosophila*. Many of these behaviors are innate, so they can be used to investigate the specific genes necessary and/or sufficient for these behaviors to occur. Perhaps the two most commonly studied social behaviors in flies are courtship and aggression.

Courtship

Flies exhibit a stereotyped courtship ritual composed of several distinct stages: (1) orientation, (2) tapping, (3) wing song, (4) licking, (5) attempted copulation, and (6) copulation. Because these behaviors are innate, scientists can use them to study the genetic basis of a hard-wired behavior. Groups of male and/or female flies can be placed into tiny chambers and recorded with a video camera. Later, an investigator scores the behavior, measuring a variety of parameters related to the timing, performance, and progression of these different stages.

Aggression

To study aggression, a scientist places two male flies in a chamber. The flies naturally fight, and the scientist can record many different parameters: which fly initiated the fight, the outcome, the interfight interval, the order of events of each fight, etc.

C. *ELEGANS* BEHAVIORAL PARADIGMS

The relatively simple and well-understood neural circuitry of *Caenorhabditis elegans*, coupled with its genetic tractability, makes this species a powerful animal model to study the genetic basis of behavior. In fact, many genes in the worm are named after the behavioral phenotypes that led to their discovery: Unc (uncoordinated) mutants have disrupted locomotion, Egl (egg-laying) mutants have disrupted egg-laying, and Mec (mechanosensation) mutants have disrupted responses to touch. Most behavioral assays in *C. elegans* examine motor and sensory functions.

Locomotor Behavior

Locomotor behavior in the worm can be automatically tracked using computer software, such as **Worm Tracker** (Fig. 2.16). This software platform and others allow for a detailed analysis of the sinusoidal movement of worms, allowing scientists to determine the genetic and cellular components that make these movements possible.

Sensory Behavior

C. elegans has proved very useful for investigating sensory transduction, as worms depend on detecting mechanical, thermal, and chemical stimuli to stay alive and find food.

Mechanosensation

A gentle touch with a fine hair to the body, head, or tail of a worm results in different behavioral responses. For example, if touched on the nose, worms

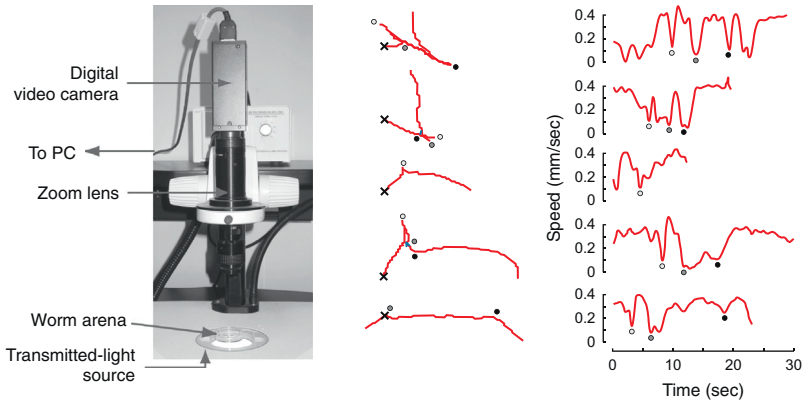

FIGURE 2.16 The Worm Tracker provides detailed information about the movements of individual worms. Representative tracks generated by the worm tracker can indicate starting position (x), path length, speed, and turning events (circles). *Reprinted from Ramot, D., et al., 2008. The parallel worm tracker: a platform for measuring average speed and drug-induced paralysis in nematodes. PLoS One 3(5), e2208, under the Creative Commons Attribution License.*

move backward. If touched on the body, worms immediately stop and sometimes move away. Because the complete circuit diagram of the *C. elegans* nervous system is known, it is possible to use these behavioral assays to map functional connections between neurons in different locations of the worm's body.

Thermosensation

Worm behavior is strongly influenced by environmental conditions—temperature, population density, and feeding status. Animals avoid temperatures at which they have been starved, and accumulate at temperatures associated with food. This association requires thermosensation, as well as a memory of the associations. There are various methods for creating thermal gradients on which to test worms: an example of a simple method is to place frozen glacial acetic acid on one end of a plate and an incubator on the other end. A more sophisticated method is to use a thermoelectric device to maintain a steady heat gradient across a plate. Investigators can then measure thermotactic migration by placing worms on the plate and identifying the temperature where migration stops.

Chemosensation

Chemosensation can be thought of as a worm's sense of taste or smell. A scientist can assay attraction to or avoidance of specific compounds by dissolving these compounds in agar and placing them on opposite sides of a Petri dish. Worms are placed at the intersection of the two chemosensory cues, and the scientist measures the number of worms that migrate to each compound.

NONHUMAN PRIMATE BEHAVIORAL PARADIGMS

Nonhuman primates can be used to study the neural basis of complex motor actions, sensory perception, and cognitive processes such as decision making, attention, and complex learning. Experiments almost always couple electrophysiological recordings with behavior so that scientists can correlate the firing of neurons with distinct behavioral events. In addition to simply measuring neural activity, it is possible to manipulate neural activity using electrical, pharmacological, and optical methods (see Chapter 8).

One of the main advantages of primates as research models is their genetic, anatomical, and behavioral similarities to humans, allowing scientists to extend results to theories about how the human brain works. Primates are also capable of more complex behaviors and tasks than rodents and other animal models. In fact, many of the specific tasks performed by nonhuman primates are the same or adapted versions of tasks performed by humans in cognitive neuroscience or psychology studies.

The most commonly used monkeys in research labs include rhesus macaques (*Macaca mulatta*) and cynomolgus (also known as crab-eating or long-tailed) monkeys (*Macaca fascicularis*). These animals can be very expensive to purchase and maintain. Therefore, each scientist typically only uses one to three monkeys over a 3- to 5-year period. While other behavioral paradigms described in this chapter typically require large numbers of individuals to achieve statistical significance, experiments involving nonhuman primates typically only need to be performed on a minimum of two animals. This limited sample size is primarily due to differences in what the scientist measures: in rodent assays, scientists typically measure quantifiable behaviors. In primate assays, scientists typically measure response properties of neurons. Therefore, statistical power is achieved not by the number of animals studied but by the number of neurons recorded.

Primate behavioral/electrophysiological experiments usually follow a specific structure: an investigator designs a behavioral task, trains an animal to perform the task, completes a surgical procedure on the animal to allow access to the brain, conducts many experimental trials while recording from neurons in the brain, and analyzes data outside of the experiment to correlate activity with specific, behavioral events.

There are no standard behavioral tasks for primates as there are for rodents and invertebrates. Each investigator designs specific tasks best suited to their own research questions, creating stimuli to maximize the response properties of the neurons to be studied. However, there are many common elements to primate experiments: they usually involve a single primate, working alone in a chamber for a juice reward, staring at a visual stimulus on a screen (Fig. 2.17). Sometimes animals are required to make eye movements to follow moving

FIGURE 2.17 A primate behavioral task. Here, the monkey has been trained to make saccadic eye movements in the direction indicated by overall movement of a random dot stimulus. The monkey fixates at a point on the screen where the motion stimulus will appear. The fixation point disappears indicating the monkey can saccade to the correct target to receive a reward. *Courtesy of Dr. Rachel Kalmar.*

dots on a screen; other experiments require monkeys to follow a path with their fingers; others require a monkey to press a lever in response to a learned cue. Like different levels of a video game, each trial within a task is slightly different, with some trials more difficult than others. Once the animal learns how to "play the game," an investigator can design dozens or hundreds of individual trials with different conditions.

Training a monkey to perform a task is often the most time-consuming aspect of an experiment. Much of this time is dedicated to simply acclimating the monkey to research life: sitting in a chair, learning how to accept a juice reward, becoming comfortable working alone in a chamber, and so forth. Most training involves operant conditioning (see Box 2.4), in which successful completion of a task is reinforced with a juice reward. Depending on the task, monkeys are trained to perform at an 80%–90% success rate. To increase success, monkeys are often food or water deprived before the task begins so they are motivated to work for juice.

During the actual experiments, a monkey performs many repetitions (trials). The number of trials performed each day is primarily determined by the monkey, who will only work for a certain amount of time before their juice is no longer perceived as rewarding. Like people, monkeys differ in the amount of work they are willing to do in one sitting. After the completion of the daily experiments, scientists return monkeys to their cages and analyze the data. Many investigators only record from primates every other day to allow time for data analysis.

CONCLUSION

This chapter has provided a framework for thinking about behaviors in commonly used animal models, surveying many specific assays frequently used in the literature. These techniques are only a fraction of the assays that investigators can use to elucidate the neural basis of behavior. Most published studies, especially studies using rodents, utilize many different behavioral assays in combination to confirm hypotheses and strengthen conclusions.

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