Chapter 10
Animal Models of Affective Behaviors and Drug Addiction

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Abstract Psychiatric disorders have a strong heritability, and like most common disorders many gene variants, each with a small effect, contribute to the genetic risk. One of the challenges in complex genetics is to determine how different alleles interact with each other and with the environment in the context of disease etiology. Here we discuss mouse models that resemble aspects of human psychiatric disorders syndromes, with particular emphasis on depression and drug addiction. These disorders rank among the top 10 causes of disability and morbidity worldwide and often occur together, thus resulting in a greater severity of health-related consequences. Mice have become the premier research organism, due to their excellent genetics. We have a large and ever-increasing number of mouse strains with defined genetic backgrounds, and the possibilities of precise genetic manipulations seem almost unlimited. Furthermore, environmental factors that are thought to influence disease outcome can be controlled, thus enabling researchers to study interactions between genes and the environment.

Abbreviations CMS: Chronic mild stress; Cnr1: Cannabinoid receptor 1; CPP: Conditioned place preference; DMS: Diagnostic and Statistical Manual of Mental Disorders; DNA: Deoxyribonucleic acid; F1: First filial generation; FST: Forced swim test; kHz: Kilohertz; OB: Olfactory bulbectomy; PR: Progressive ratio; QTL: Quantitative trait loci; RI: Recombinant inbred; SSR: Simple sequence repeats; SSRIs: Selective serotonin reuptake inhibitors; STR: Short tandem repeats; TST: Tail suspension test; USV: Ultrasonic vocalisation; VNTR: Variable number tandem repeats

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C.W. Turck (ed.) *Biomarkers for Psychiatric Disorders*,
doi: 10.1007/978-0-387-79251-4_10,

10.1 Introduction

Mice have become the premier vertebrate research organisms because they are readily amenable to a wide spectrum of genetic manipulations (Dierich and Kieffer, 2004; Ristevski, 2005; Brault et al., 2006; Abuin et al., 2007; Gaveriaux-Ruff and Kieffer, 2007; Schmidt-Supprian and Rajewsky, 2007). We can introduce new genes, delete or replace genes, or introduce tissue-specific mutations. Using dual systems that consist of a gene locus that can be activated or changed and specifically engineered transcription factors or DNA recombinases, we can induce gene alterations by administering drugs such as tamoxifen or tetracycline. Genetic manipulations of the murine genome can be restricted to one specific gene, or it can encompass larger chromosomal regions and thus affect many genes. Indeed it is possible to exchange or delete large chromosomal regions. In addition, we can generate allelic series of point mutations with sophisticated methods for chemical mutagenesis and mutation screening. Genetic modifications of different kinds are already available for at least one quarter of all murine genes, and soon we will probably have a mouse mutant for every gene. We can also induce genetic manipulations in specific organs of adult mice with viral vectors (Aronoff and Petersen, 2006; Pfeifer, 2006). And we can do even more than that. We can label cells, organelles or proteins with fluorescent markers (Ristevski, 2005). We can visualize gene activity with reporter genes. We can manipulate ion channels in such a manner that they are activated by light of specific wavelength, thus turning neurons on and off with diodes of different colors (Zhang et al., 2007). In other words, no other mammalian organism comes even close to the diversity of genetic models that we now have available for mice. Such genetically modified mice have already contributed enormously to our understanding of the biological basis of psychiatric disorders (Takao et al., 2007). Although they may not have as highly developed cognitive and social functions as rats or monkeys, mice are used to investigate specific signaling pathways or specific neuronal circuits in normal brain physiology and in pathological mechanisms.

Psychiatric disorders have a strong heritability and, like most other common disorders, they are complex (Meyer-Lindenberg and Weinberger, 2006). This means that these disorders are caused by a combination of different common gene variants, whereas a single gene only contributes a small percentage to the overall genetic risk load. Thus they can be contrasted to the less frequent Mendelian disorders, where one gene mutation causes a disease phenotype. Genes involved in Mendelian disorders are readily identifiable, provided researchers have access to samples (e.g., families with affected and unaffected individuals) that are sufficiently large or informative enough for genetic mapping. In contrast, it has been a rather daunting task to identify the underlying gene variants in complex disorders. The most successful studies so far have used candidate gene approaches where the biological evidence already suggested a possible involvement in the disease process. Only with the advent of chip-based technologies that permit the detection of hundreds of thousands of polymorphisms in each individual, has it been possible to

realize systematic whole-genome association studies (Fan et al., 2006; Eberle et al., 2007; Ropers, 2007). Nevertheless, these studies are still daunting and cost-intensive, because they ideally require very large sample sizes with more than 10,000 cases and controls. Currently, we know very little about complex genetics and how common gene variants contribute to disease etiology. Common gene variants often do not affect the protein-coding capacity of a gene locus and thus are thought to change gene regulatory mechanisms. Indeed, disease-associated alleles are often unknown, because association studies only point out specific polymorphisms or haplotypes that are over- or underrepresented in the case vs. control sample. It then remains to be determined which of the observed polymorphisms is responsible for the increased genetic risk – a task that has not often been met with success so far. These small and subtle gene effects are difficult to address with genetically manipulated mouse strains. Although it is possible to generate "humanized" mice that carry gene regulatory alterations in disease-associated alleles, it remains to be shown how informative such mouse models will be (Shultz et al., 2007). Nevertheless, mouse geneticists have used "classical" genetics to breed a large number of diverse inbred mouse strains with interesting phenotype characteristics that may be relevant for human psychiatric disorders. Such mouse strains resemble the human situation and can be exploited to study specific questions related to complex phenotypes. The importance of this resource has been recognized and currently a large effort to create over 1,000 such diverse inbred strains is being funded by the Collaborative Cross project.

In complex genetics, one of the most challenging tasks is to determine how different genes interact with each other and with the environment in the context of disease etiology. One possibility of gene-gene interactions is the simple additive effects and a more or less linear relationship between genetic risk load and disease probability. However, evidence from chromosome substitution strains indicates that this is not the case. Rather it seems that most gene-gene interactions are synergistic and certain gene combinations carry a risk load that is far greater than the sum of the individual effects. Other gene combinations have the tendency to cancel each other (Nadeau et al., personal communication). In addition, environmental factors can lead to the activation of transcription factors by intracellular signaling cascades and concomitant expression changes. These events usually are of rather short duration and typically last for no more than days or weeks, but they may also result in long-term alterations through epigenetic modifications of the chromatin structure. Thus, events during early childhood may affect the gene regulatory processes of the adult organism. Mice offer the great advantage that environmental factors can be easily controlled and varied. Numerous studies have already shown that such environmental variables have a profound influence on the expression of behavioral phenotypes (and others) (Tsankova et al., 2007). Elucidation of these complex effects probably requires systems approaches including expression profiling, proteomics, epigenomics, etc. (Sieberts and Schadt, 2007). The evaluation of animal behaviors is also important for psychiatric phenotypes.

The term "behavior" generally refers to the way in which an organism acts under specific conditions or in relation to specific circumstances. Behavior represents the

final integrated result of genetic, biochemical, physiological and neuronal processes. Thus, behavioral responses may be highly sensitive indicators for environmental or physiological changes. However, they are rarely informative about specific pathways that contribute to the behavioral change. In other words, behavioral testings often tell us that something is different with an animal, but not why. Behavioral testings are commonly used in functional neuroscience studies, drug development, or environmental monitoring, e.g., in exploring environmental toxicants (Wurbel, 2002; Wahlsten et al., 2003; McArthur and Borsini, 2006). A behavioral test always has one or several dependent variables that correspond to the behavioral readout. Animal models also have independent variables that induce specific disease-related conditions, such as genetic manipulations, surgeries, exposure to specific environmental factors, or drug administration. There is an ongoing debate about the validity of animal models for human psychiatric disorders that extend to virtually every aspect of animal experimentation including the conceptual, theoretical framework, the suitability of the species, or the usefulness of pharmacological vs. genetic interventions. This debate is often fuelled by the fact that the behavioral responses in such studies are used as an indicator for emotional or cognitive aspects that cannot be directly addressed in experimental animals.

An animal model that resembles a human clinical condition should meet three validity criteria (Sarter and Bruno, 2002; Willner and Mitchell, 2002):

- Construct validity refers to the degree of overlap between the cause or rationale of the human condition and the animal model.
- Face validity reflects the extent to which the animal behavioral responses reflect human symptoms.
- Predictive validity indicates the value of an animal model to predict the efficacy of pharmacological treatments in humans.

In this chapter, we address the strengths and weaknesses of behavioral paradigms by describing experiments related to affective disorders and drug addiction. Drug addiction and depression rank among the top 10 causes of worldwide disability and morbidity in human populations (Murray and Lopez, 1996). These disorders often occur together, thus resulting in a greater severity of health-related consequences such as an increased number of suicides. The reason for the high degree of comorbidity is poorly understood. Many investigators favor the view that one is a cause or consequence of the other, but there is also an increasing body of evidence suggesting that both disorders share some etiologies and pathomechanisms (Paterson and Markou, 2007). Thus, drug addiction and depression are modulated by partially overlapping neuronal circuits (e.g., reward system), neurotransmitter systems (e.g., serotonin, endocannabinoids), hereditary factors (e.g., Cnr1 polymorphisms), and environmental influences (e.g., stress). Many of the diagnostic criteria for depression and drug addiction involve subjective emotional symptoms that cannot be directed in mice. It is thus difficult, if not impossible, to device a behavioral paradigm that meets all validity criteria. Indeed, it is highly questionable if mice can experience emotions that resemble sadness in humans. In practice it is sometimes difficult to evaluate, based on behavioral responses alone, whether symptoms

such as reduced locomotor activity or anhedonia have a neuronal cause, or whether a mouse is simply sick. Nevertheless, there are a number of animal models that recapitulate certain aspects or symptoms of depression. This chapter is by no means intended to provide the uninitiated reader with precise experimental protocols. We are rather using these paradigms to demonstrate some general issue that should be considered for the assessment and interpretation of behavioral phenotypes.

10.2 Behavioral Despair Tests

Behavioral despair paradigms are based on the observation that rodents placed in an uncomfortable and inescapable situation initially show escape behaviors followed by periods of inactivity, which was assumed to indicate that the animals had given up hope of escaping (behavioral despair) (Katz, 1982). Antidepressants increase the duration of escape behaviors and, conversely, decrease the duration of immobility (Steru et al., 1987). Immobility in this test is not an indication of hypomobility; it rather reflects the animals' reluctance in continuing the escape attempts. It might represent a correlative to the observation that patients suffering from depression also show deficits in psychomotor tests that require a sustained effort. These paradigms have a high degree of predictive validity, because they are sensitive for all major classes of antidepressant treatments: Monoamine oxidase inhibitors, tricyclic compounds, and selective serotonin reuptake inhibitors (SSRIs), as well as electroconvulsive shocks show efficacy in this test (Borsini and Meli, 1988). The test is relatively simple and reproducible between different laboratories, therefore it is commonly used as a screening test by the pharmaceutical industry. It should be noted that acute drug treatments are effective in these assays, although antidepressant effects in humans are only observed after repeated or prolonged use.

In the forced swim test (FST, Porsolt's test), developed by Porsolt and co-workers, a mouse is placed in a water-filled (23–25°C) cylinder from which it cannot escape (Porsolt et al., 1977a,b). The depth of the water has to be sufficient (at least 10 cm) so that the animal is unable to make contact with the bottom, not even with its tail. Most mice can easily float on the surface and require only slight movements to keep the head above the water. Typically, mice initially show a period of escape-orientated movements in the FST, followed by a period of predominant immobile postures. The duration of this test is usually 5–6 min of which only the immobility time during the last 4 min is scored (Lucki et al., 2001).

Steru and colleagues developed another behavioral despair paradigm called the tail suspension test (TST) (Steru et al., 1987). Here, mice are suspended by their tails for 5–6 min and their immobility time is scored. Automatic scoring devices have been developed for these tests which allow high throughput screens and the evaluation of additional Parameters such as force, power and energy of movements. FST and TST are conceptually very similar, but influenced by different confounding variables. Thus, a disruption of thermoregulatory processes can affect FST responses, while TST is more sensitive to effects on motor functions. Indeed, some

mouse strains, including the commonly used C57BL/6J mice, are known to climb up on their tails and cannot be used in the TST assay (Dalvi and Lucki, 1999). Both tests therefore provide complementary or converging information.

10.2.1 Distress Vocalization

When newborn rat and mouse pups lose contact with their littermates or mother, they emit calls in ultrasonic frequencies (30–90 kHz) that induce a searching behavior in the mother. In addition to the absence of olfactory and tactile cues, cold also elicits the calling behavior (Branchi et al., 1998). The ultrasonic vocalization test (USV) in rodent pups is an ethologically valid model for the measurement of emotionality because it correlates with stress reactivity. Antidepressants (de Paulis, 2007) and anxiolytics reduce the duration and number of calls, whereas anxiogenic agents induce changes in the opposite direction. This procedure is frequently used to test altered emotionality in genetically modified mice (Winslow et al., 2000; Santarelli et al., 2001; Scattoni et al., 2007). In mice, the calling reaches a peak at postnatal days 6–8 and disappears during the second week after birth (Branchi et al., 1998). Therefore, it is critically important to precisely match the age of control and test animals. The experimental design of the USV test is quite simple: The pups are placed on a cold (16–20°C) glass jar in a sound-isolated chamber containing an ultrasonic detector. The test typically lasts for 10 min and requires specialized software for the analysis of the calls.

10.2.2 Learned Helplessness

This model was originally developed for dogs and later adopted for rats and mice (Overmier and Seligman, 1967; Seligman and Beagley, 1975). It involves the exposure of mice to inescapable mild footshocks. Such animals may show escape deficiencies when re-exposed to a similar situation with an escape opportunity. It has been demonstrated that a broad spectrum of antidepressants increases the frequency of escape attempts, although the efficacy of different compounds varies substantially between different strains of mice. One major caveat of this model is seen in the fact that only a certain percentage of mice (between 10 and 80% depending on the mouse strain) show the learned helplessness behavior (Cryan and Mombereau, 2004). The 129 mouse strain, from which most embryonic stem cells used for gene targeting are derived, unfortunately seems to perform particularly poorly in this test. Indeed, even non-shocked 129 control mice show a poor escape performance. Thus, it is important to ensure that mice with targeted gene deletions have been crossed to a different strain and contain no residual of the 129 genetic background that may affect the test results. The model is also sensitive to gender effects. Male mice normally show a stronger learned helplessness behavior than females (Caldarone

et al., 2000). This model is also influenced by any alterations in pain sensitivity. Thus, genetic or drug effects on nociception that may result in a reduced learned helplessness should be excluded.

10.2.3 Chronic Mild Stress

A number of animal models for depression are based on the exposure of animals to repeated stressors. Many investigators feel that these models offer a high degree of face validity, because episodes of major depression often occur in the context of stress. Katz and collaborators developed a rat model in which animals are exposed to a series of intense stressors (Katz, 1982), which was modified for mice by exposing animals to various relatively mild and unpredictable stressors (Willner et al., 1987). In this chronic mild stress (CMS) model mice are exposed to environmental stressors like tilted cage, food or water deprivation, paired caging, overnight illumination or wet bedding during a prolonged period of usually more than 2 weeks. This chronic mild stress induces long-lasting behavioral and neurochemical changes, which parallel aspects of depressive disorders in humans (Willner, 2005). Behavioral changes in mice are seen in a reduced sensitivity to rewards, termed anhedonia. Anhedonia is, according to Nelson (Nelson and Charney, 1981), a key symptom of the human depressive state and concerning DMS IV (Association, 1994) a core feature of the melancholic subtype. It also has good predictive validity, as anhedonia can be reversed by chronic treatment with several classes of antidepressants (Willner, 1995; Papp et al., 1996). However, this model has a number of disadvantages that limit its widespread use. The CMS model has a very poor reliability across different laboratories and often cannot be reproduced, even in laboratories where it had been successfully established before.

10.3 Bulbectomy

In this model, the olfactory bulbs of adult mice are bilaterally removed via a microsurgical procedure (olfactory bulbectomy, OB). About 2 weeks after surgery, mice show aspects of behavioral alteration, such as hyperactivity in the open field, disturbed eating patterns, anhedonia or memory deficits. The most commonly evaluated behavioral change in this model is hypermotility in the open field (Zueger et al., 2005). These changes are thought to originate from a complex pattern of neurochemical, neuroendocrine and neuroimmune alterations representing compensatory-neuronal-reorganization and alterations in synaptic connections (Jarosik et al., 2007). These changes in synaptic plasticity are noticed in various subcoritcal limbic regions such as the amygdala and hippocampus and are qualitatively similar to those observed in depressed patients (Cryan and Mombereau, 2004; Song and Leonard, 2005). Hence, like the CMS model, the OB model

possesses high face validity. Most changes occurring after OB can be attenuated or reversed by prolonged antidepressant treatment (Otmakhova et al., 1992; Kelly et al., 1997; Song and Leonard, 2005).

10.4 Depression and Drug Addiction

Depression is a common comorbidity with drug addiction (Robbins, 1974) and also occurs frequently during abstinence. Both withdrawal from substance abuse and depression have been linked to changes of several neurotransmitter pathways including serotonin, dopamine and glutamate (Palomo et al., 2007). Drug addiction is a complex brain disorder characterized by an inability to control drug use, despite severe adverse effects. The addiction process can be divided into different stages including controlled use, compulsive use, abstinence and relapse. Chronic substance use/abuse is associated with physiological adaptations, such as the development of drug tolerance. The mechanisms that trigger the transition from controlled use to addiction are still largely unknown. Most addiction processes can be studied in mice, as they readily self-administer almost all drugs abused by humans, show adaptive changes after prolonged drug exposure and withdrawal symptoms as well as drug-craving behavior. Thus, animal models have been developed to assess almost all aspects of drug addiction including acute effects (e.g., thermoregulation, motility), tolerance, somatic drug dependence, drug preference and reward and relapse to drug-seeking behavior.

10.5 Drug Self-Administration/Drug Preference

Models that evaluate whether an animal finds a drug rewarding include self-administration and place conditioning paradigms. For substances like ethanol and nicotine, the two-bottle choice paradigm is often the method of choice to evaluate drug preference via oral self-administration (Fig. 10.1). In such experiments the animals have access to a drug-solution and water, which may contain flavors to match the taste of the drug solution. To exclude a positional bias, the position of the bottles is regularly alternated and the proportion of drug consumption relative to total fluid intake is calculated as a preference ratio. Liquid consumption is mostly evaluated by weighing the bottles, although some companies are developing automated devices. Albeit this paradigm is mostly used for alcohol and nicotine, it has also been used with other drugs such as cocaine, barbiturates and benzodiazepines (Meisch et al., 1992; Stewart et al., 1994; Jentsch et al., 1998). It can be argued whether this model has good construct validity. However, the motivation for drug use is certainly different between animals and humans, as social factors (e.g., peer pressure) contribute probably at least as much as the reinforcing properties of the drug during the early stages of drug consumption.

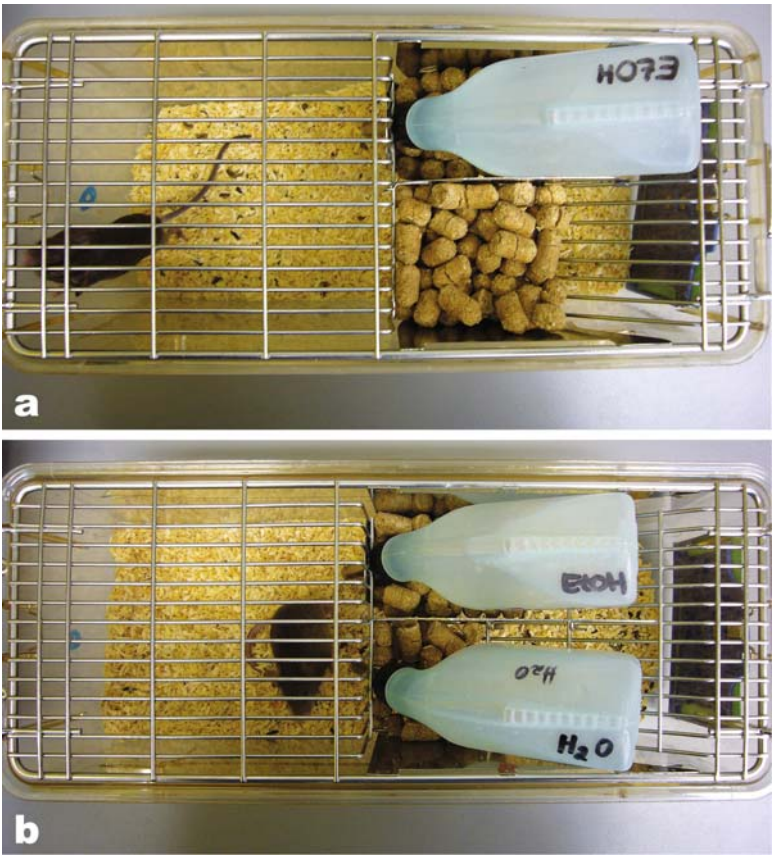

Fig. 10.1 *Forced drinking* (**a**) and *Two bottle-choice paradigm* (**b**). In the *forced drinking paradigm* the animals are forced to consume distinct amounts of drug-solution over an extended period of time. Contrastingly, in the *two bottle-choice paradigm* the animals have the choice between a drug-solution or tap water to evaluate drug preference (*See Color Plates*)

Operant self-administration paradigms were originally developed by Skinner in the 1930s. It involves an apparatus (Skinner box) equipped with one or more operandi (typically a lever or a sensor) that can register behavioral actions and activate the delivery of a reinforcer. The paradigm is based on the concept that behaviors are driven by their outcome. Thus, delivery of a positive reinforcer will strengthen the behavioral response.

Some drugs of abuse have aversive properties that may limit the drug intake and thus the transition to drug addiction. Different protocols were established to minimize these aversive effects. For ethanol, the saccharin fading protocol is a widely

used model. Here, the concentration of ethanol in a mixed ethanol/saccharin solution is slowly increased until ethanol is presented alone (Hansson et al., 2007). Different schedules of reinforcement exist to evaluate self-administration behavior in animals. In the fixed ratio schedule of reinforcement, the reward (food, drugs) is delivered after a fixed number of responses (lever presses) have been made.

In operant behavioral tasks like the progressive ratio (PR) test, the instrumental demand for a constant reward increases progressively until responding ceases and reinforcers are no longer obtained. This "break point" is an operational measure for a shift in motivation, where the rewarding value is lower than the effort the animal is willing to expend to obtain it. The PR test is a complex task, where animals are forced to adjust their instrumental behavior from continuous reinforcement to an incremental schedule of responding. This task implies switching from a fixed ratio to an increasing schedule and the dynamic adjustment of behavior according to a cost-benefit analysis. Therefore, it is useful for testing brain mechanisms underlying the translation of motivation and changing values of reinforcement into appropriate behavior (Drews et al., 2005). In addition to oral intake, reinforcers are also administered directly into brain regions of interest.

Replacement of the drug-reinforcer with a neutral substance results in a gradual extinction of the operant behavior. These behaviors can, however, often be reinstated by factors that trigger relapse in humans, such as drug-associated cues, priming with a small amount of the drug, or stress-exposure. This experimental design thus permits the analysis of mechanisms that lead to the relapse of drug-seeking behaviors after a period of abstinence.

Probably the most common model to study the motivational effects of drugs of abuse in experimental animals is the conditioned place preference (CPP) paradigm. Genetically modified animals (knock-out and transgenic animals) are used in the CPP to analyze gene functions in drug reward. Furthermore, drug tolerance and sensitization, as well as extinction and reinstatement procedures, can be examined by means of the CPP paradigm (Tzschentke, 2007).

The CPP paradigm uses a classical conditioning procedure for which a variety of protocols have been adapted to meet specific conceptual requirements. In a simple version, animals are exposed to two or more distinct neutral environments such as two boxes or compartments provided with different floors, marked walls, colors, texture or lighting (Fig. 10.2). First, a potential place bias is determined in an initial session where animals can freely explore the entire apparatus. During the subsequent conditioning phase, animals are alternately restricted to one or the other compartment, where they receive either a drug or a control solution. The environments are thus paired with distinct drug or non-drug states. In a final test session, animals have again the opportunity to move freely in the entire apparatus. If the drug is reinforcing, animals will now spend more time in the drug-associated compartment when compared to the initial session. Conversely, if the drug is aversive, the animal will spend more time in the non-drug associated compartment. The CPP paradigm thus provides indirect information about the positive and negative reinforcing effects of a drug (Carboni and Vacca, 2003).

Fig. 10.2 *Conditioned place preference paradigm*. Animals are exposed to two or more distinct neutral environments that are differentiated from each other. After several conditioning sessions, this paradigm provides information about positive and negative reinforcing effects of a drug (*See Color Plates*)

10.6 Drug Tolerance and Sensitization

Tolerance is defined as a state of progressively decreasing drug response so that increasing drug dosages are needed to produce the desired effect. Two ways of tolerance have been examined, viz., acute and chronic tolerance. Acute tolerance is measured after one or two drug exposures within the same testing session, whereas chronic tolerance is measured after repeated drug exposure (Bennett et al., 2006).

Depending on the drug, there are several ways to determine tolerance in animal models. Measurement of drug-induced hypothermia for example is a commonly used method to determine chronic tolerance to ethanol in rodents. Therefore, drug–naïve

mice receive an intraperitoneal injection of an ethanol solution that leads to a measurable loss of body temperature. Most mice will develop a tolerance to the hypothermic effects of ethanol after chronic ethanol administration over a period of several weeks. Thus, the ethanol-induced hypothermia is reduced after chronic exposure. Because of its antinociceptive properties, tolerance to morphine is usually evaluated by the reduction or loss of analgesia after chronic morphine treatment.

On the other hand, some drug effects increase after repeated drug exposure. This sensitization phenomenon is typically observed as an increased locomotor activation after repeated psychostimulant administration. Conceptually, it is sometimes considered an example of non-associative learning in which the gradual strengthening of a response follows repeated administrations of a stimulus (e.g., drugs) (Bell et al., 1995). Sensitization has also been implicated in the vulnerability to relapse. Despite researchers having already found several factors to be associated with the development of sensitization, there is no complete understanding of when tolerance or sensitization will occur after chronic drug use. Factors that are involved in drug sensitization processes include genetic variations of the subjects (Phillips, 1997), the schedule of drug administration (Robinson and Becker, 1986), and the novelty of the environment (Robinson and Berridge, 2000). Some studies have showed that animals more likely become sensitized to a drug if it does not inhibit their goal-directed behavior, e.g., food intake (Wolgin and Hertz, 1995; Pinkston and Branch, 2003). On the other hand, development of drug tolerance often appears if drug-related behaviors (locomotor activity, anxiety) interfere with such goal-directed behaviors (Wolgin and Hertz, 1995).

10.7 Drug Dependence and Withdrawal

Withdrawal follows cessation of chronic drug consumption or treatment in dependent individuals. It produces several symptoms that may provide markers for the study of neurobiological mechanisms of dependence (Bennett et al., 2006). Existing standard rating scales help to easily quantify many of the physical signs occurring after withdrawal of several substances (opiates, nicotine and alcohol).

Mice will become physically dependent on ethanol if they are forced to consume significant amounts over an extended period of time (forced drinking paradigm, Fig. 10.1). When ethanol is removed, such mice will display withdrawal symptoms that may start within hours and last for several days. These symptoms typically include handling-induced convulsions, excitation and increased anxiety-like behaviors (Mayo-Smith, 1997; Watson and Little, 1999). Animal models for these behaviors possess good face validity for human withdrawal, also including tremors, excitation, seizures and anxiety (Kosten and O'Connor, 2003). Ethanol withdrawal – induced convulsions or seizures are easy to measure via turning and lifting the animals and are scored by severity (Watson et al., 1994).

To evaluate physical withdrawal in rodents dependent on opiates, withdrawal symptoms can be induced by the administration of opioid antagonists such as

naloxone. After injection of the antagonist, the animals exhibit typical behaviors like wet-dog shakes, tremors, chews, teeth-chattering, diarrhea, etc., that, again, can be quantified by scores. The same approach is often used to determine symptoms of nicotine withdrawal. After administration of a nicotinic antagonist (e.g., mecamylamine), the animals display body shakes, chews, tremors, yawns and scratches. The frequency and time of occurrence is recorded and quantified.

In addition to the physical signs, there are the motivational aspects of drug withdrawal. Measuring these aspects may help to understand the counteradaptive mechanisms that may drive addiction. Animal models for motivational aspects of drug withdrawal include operant schedules (Gellert and Sparber, 1977), place aversion (Stinus et al., 2000), intracranial self-stimulation (Schulteis et al., 1995) and drug discrimination studies (France and Woods, 1989).

10.8 Relapse

Drug addiction is a chronic disorder and relapse to drug addiction can occur even after prolonged periods of abstinence. The most common reasons why humans revert to addictive behaviors include exposure to drug associated cues, drug priming and stress. Conditioned cues acquire reinforcing properties on their own, as they signal the availability of a reinforcer and thus predict reward. Generally it is believed that the same neuronal mechanisms involved in drug reward are also involved in relapse. There are two main theories about how reward pathways mediate relapse to drug-seeking. One postulates that drug reward pathways overlap with relapse pathways (Self and Nestler, 1995; Robinson and Berridge, 2000). The other suggests that an opponent process produces a hypofunctional state of the reward circuitry, which in turn leads to dysphoria or anxiety during withdrawal (Koob et al., 1997). Following this theory, drug-seeking is the attempt of alleviating the aversive feeling of withdrawal.

To assess reinstatement of drug-seeking behavior in mice, the subjects first have to acquire an operant self-administration behavior, followed by an extinction phase. For reinstatement, animals are exposed to conditioned cues (typically a light, smell, or tone), to a small dose of the drug, or to an external stressor (de Wit and Stewart, 1981). Reinstatement is indicated by a preferential activation of the reward-associated operandum, even when it is not rewarded by a drug delivery.

10.9 Analytical Tools

Drug addiction-related behavioral phenotypes in a genetically heterogeneous mouse population are influenced by multiple gene loci, each of which typically contributes to a small effect. Thus, the behaviors vary by degrees and constitute a quantitative trait. Genetic loci contributing to these quantitative traits (quantitative trait loci, QTL) are identified by quantitative genetic approaches (QTL analysis).

Two common strategies are used in mouse QTL studies (Belknap et al., 2001). The fist strategy is based on the breeding of a suitable genetically diverse population of mice, followed by phenotype and genotype analyses of the individuals. Typically, two or more inbred strains of mice are crossed for at least two generations. The progenitor mouse strains should have sufficient variation for the traits of interest and they should be genetically diverse enough to enable genetic mapping (Flint, 2003). The sample size required for the identification of QTL depends largely on the size of the effect that a QTL contributes to phenotypes on interest. Inferences about QTLs can be made if one or more genetic markers are overrepresented (or underrepresented) in individuals scoring in the low or high range of the phenotype spectrum. Genotyping is often done with the use of microsatellite markers, which contain mono, di, tri, or tetra tandem repeats flanked by specific sequences. Such simple sequence repeats (SSR), short tandem repeats (STR), or variable number tandem repeats (VNTR) have been characterized for most commonly used inbred mouse strains.

The second strategy involves the phenotyping of recombinant inbred (RI) strains, which have a unique combination of well-circumscribed genome contributions from two or more progenitor inbred strains. Traditionally, RI strains have been derived by consecutive brother x sister matings (>20 generations), starting from an F1 generation of an intercross of two inbred strains. More advanced RI strains, as those generated in the Collaborative Cross project, are derived from multiple inbred strains.

10.10 Summary and Perspective

In this chapter, we have summarized some mouse models for the analysis of behaviors related to depression and drug addiction, two important complex brain disorders that often occur together. Both disorders are characterized by a spectrum of symptoms, including emotions that may be uniquely human. They are also influenced by a number of social and environmental factors that are irrelevant for mice. Thus, we cannot build rodent models for addiction and depression that reflect all facets of the human condition. Nevertheless, we have a number of mouse models with excellent validity for specific aspects or symptoms of these disorders, which we can utilize to study the neuronal circuits and cellular-molecular mechanisms involved (Arguello and Gogos, 2006). We believe that these findings will result in novel mechanism-based pharmacotherapies for psychiatric disorders.

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