Chapter 9
Animal Models for Anxiety Disorders

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Abstract Anxiety disorders are characterized by overwhelming anxiety or fear and are chronic and relentless if left untreated. Current available treatments for anxiety disorders are inadequate and some have severe side effects, thus warranting a better understanding of the etiology and mechanisms underlying anxiety and the development of anxiety disorders. In this chapter, the use of animal models to identify molecular and cellular circuitry that regulate fear or anxiety, and the influence of environment on the development of fear or anxiety, are discussed.

Abbreviations ACTH: Adrenocorticotropin; CORT: Corticosterone; CRH: Corticotrophin-releasing hormoneCS: Conditioned stimulus; 5-HT1A: Serotonin 1A receptor; LTP: Long-term potentiation; MAO-A: Monoamine oxidase A; PTSD: Post-traumatic stress disorder; SSRI: Serotonin reuptake inhibitors; US: Un-conditioned stimulus

9.1 Introduction

Anxiety disorders affect approximately 19 million American adults, and are characterized by overwhelming anxiety and fear. These disorders are chronic, relentless, and can grow progressively worse if not treated. In this chapter, I will first give an overview of various anxiety disorders that are observed in humans. Then I will discuss the research on the underlying mechanisms, which looks into the pharmacology, neural circuitry, genetics, and environmental effects, using various paradigms of fear/anxiety in animal models. Since a majority of currently available paradigms have employed rodent models, they will be discussed most extensively. Other animal

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models including primates and zebrafish will also be described, wherever data are available. Finally, the impacts of the studies in animal models on future improvement of diagnoses, treatments, and prevention of human anxiety disorders will be discussed.

9.2 Overview of Human Anxiety Disorders

While the relatively mild and brief episodes of fear or anxiety are often protective and beneficial, uncontrollable and persistent emotional and physiological symptoms, as manifested during anxiety disorders, are detrimental to the health and well-being of an individual. These symptoms further pose huge economic burdens on the family and society. Six forms of anxiety disorders have been described in humans: (1) Panic disorder is characterized by unpredictable, rapid-fire attacks of intense anxiety. (2) Generalized anxiety disorder is marked by exaggerated worry and tension, even though there is little or nothing to provoke it. (3) Social phobia, also called social anxiety disorder, involves overwhelming anxiety and excessive self-consciousness in daily social situations. (4) Specific phobias are intense fears of some specific objects that in reality pose little or no danger. (5) Obsessive-compulsive disorder involves anxious thoughts and repetitive rituals, which, when performed, could help reduce anxiety. (6) Post-traumatic stress disorder (PTSD) is characterized by persistent frightening thoughts and memories of a terrifying event.

Currently, two types of treatment are available for anxiety disorders: medication and specific types of psychotherapy. Although these treatments are effective in some people, many patients are left with residual symptoms or experience side effects that limit the use of these currently available mediations. Therefore, improved understanding of the molecular and neural basis of anxiety and anxiety disorders could potentially lead to improved treatments.

9.3 Animal Models of Anxiety Disorders

In order to screen for therapeutic compounds and to understand the molecular and neural basis of anxiety and anxiety disorders, animal models have been extensively employed. Indeed, the ability to respond to a threat or danger is universal in the animal kingdom. Since adequate responses are essential for the survival of the individual and species, these responses have been evolutionarily selected. Animals respond to the potential presence of a threat with characteristic autonomic and behavioral responses that are quantifiable. Through the analysis of innate or learned anxiety-like behaviors in animals at the pharmacological, molecular, genetic, and neural circuit levels, mechanisms that regulate anxiety-like behaviors have begun to emerge.

In the following paragraphs, paradigms that assess either innate anxiety- or learned anxiety- like behaviors in animals will be discussed. Their potential relationship to specific forms of human anxiety disorders is suggested. However, like animal models of any human diseases, the quality of an animal model is typically assessed by three validity criteria: Face validity describes how closely the animal model mimics the key symptoms of the human disorder; predictive validity refers to the success in the animal model of a pharmacological treatment, which is shown to have therapeutic effect in humans; and construct validity states that the cellular and molecular processes in the animal model and the human disorder are analogous (Willner, 1984). In the case of animal models of anxiety disorders, wherever the data are available, these criteria of validation will be discussed.

In addition to modeling human anxiety disorders for the purpose of pharmaceutical drug screening and testing, animal models of anxiety and anxiety disorders provide an opportunity to identify the environmental and genetic factors that control the neuronal circuitry, which in turn underlies the regulation of fear or anxiety.

9.3.1 Models for Generalized Anxiety and Panic Disorders

9.3.1.1 Assessing Innate Anxiety-Like Behaviors

Test of Exploratory Behavior

Many of the currently used models use animals' exploratory behavior as an index of anxiety. It has been proposed that the behavior of animals exposed to a novel situation results from a competition between an exploratory tendency (motivated by curiosity or boredom) and a withdrawal tendency (motivated by fear or anxiety) (Montgomery, 1955). Specific behavioral paradigms that employ animals' exploratory behavior to assess anxiety include the following: (1) The open field assay, which is popular because it requires minimal apparatus and is quickly performed (Walsh and Cummins, 1976). In this assay, locomotion and defecation are often used as indices for measurement. However, the use of locomotion as an index of anxiety is complicated by the fact that mechanisms that change locomotion do not always involve changes in anxiety. Although defecation can be used as an indication of the activation of the autonomic nervous system, food intake or drugs that alter gut motility, rather than anxiety, could affect defecation. Therefore, a better modification of the open field assay, namely "novelty-suppressed feeding," which assesses the animals' approach to the consumption of food located in the center of the open field, appears to be a more sensitive measure of individual differences in emotionality from a psycho-behavioral perspective (Britton and Brittone, 1981). (2) The plus-maze test, which was initially developed by Montgomery (Montgomery, 1955). Pellow and co-workers performed studies to validate it as an animal model of anxiety in rats (Pellow et al., 1985), and Lister validated the test in mice (Lister, 1987).

This test has proved very popular in recent years, in part because it is both rapid and appears to be sensitive to the effects of both anxiolytic (benzodiazepine, barbiturates, and ethanol) and anxiogenic (benzodiazepine receptor inverse agonists, caffeine, and yohimbine) agents. The elevated plus-maze test also has good face validity, in that the reluctance of animals to explore the open arms of the maze probably results from a combination of their aversion to open space and the elevation of the maze. 3) Light-dark transitions, which was initially developed by Crawley and colleagues (Crawley and Goodwin, 1980). This test also possesses a certain degree of face validity, in that light serves as an anxiogenic stimulus, and there is an apparent conflict between the desire to explore and the desire to avoid the brightly lit part of the apparatus. A number of benzodiazepines and meprobamate produce anxiolytic profiles; however, pentobarbital fails to alter the number of transitions at doses that did not increase activity (Crawley and Goodwin, 1980).

Tests of Social Behavior

In addition to testing exploratory behaviors as a way of measuring innate anxiety, assessment of social behaviors, in which aggression is infrequently observed, has also been used to measure innate anxiety. The social interaction test, developed by File and colleagues, uses novelty and high lighting conditions as anxiogenic stimuli (File, 1988). The time spent by pairs of male rats in active social interaction is measured in a test arena under high or low lighting conditions, and/or in a familiar or unfamiliar testing apparatus. Locomotor activity is also measured, and this assists in determining whether any change in social interaction is due to a general stimulant or sedative effects. As in the plus-maze test, when both the time spent in social interaction and locomotor activity are affected by a treatment or a genetic condition, analyses of covariance are performed with locomotor activity as a covariate. Attributing the reduction in social interaction to increased anxiety in this test appears to have some face validity. Ethological studies suggest that rats find high light and novel environment aversive. Moreover, plasma corticosterone levels are higher in rats that are subject to high light conditions or in an unfamiliar environment (File and Peet, 1980). An anxiolytic profile of benzodiazepine is observed following chronic treatment after tolerance has developed to the drug's sedative effects. The benzodiazepine increases the amount of time of interaction under conditions of high lighting or in unfamiliar arena. Other anxiolytics, such as phenobarbitone, have been found to increase social interaction across all test conditions (File and Peet, 1980). Anxiogenic agents appear to reduce the time spent in social interaction without altering locomotor activity. A test of social interaction has been adopted in mice, but is met with a challenge, in that pairs of mice have a tendency to be much more aggressive during the test than pairs of male rats (Lister and Hilakivi, 1988). A disadvantage of the social interaction test is that the test involves the observation of pairs of animals, thereby making it difficult to examine individual difference in behavior with this test, as the behavior of one animal is critically dependent on the behavior of its test partner. Nevertheless, this test may shed light on social phobia, which is a type of anxiety disorder observed in humans.

The ultrasonic vocalization emitted by rat pups following separation from their mother has also been used as an animal model of anxiety (Gardner, 1985). The method has some face validity since the ultrasonic cries are considered to be "distress" calls (Noirot, 1972). One needs to keep in mind, however, that ultrasonic calls are also emitted in response to cold and to tactile stimulation resulting, for example, from retrieval by the mother, or contact with unfamiliar surfaces (Okon, 1972). Therefore, it is important to examine whether any change in ultrasonic calls is secondary to some alteration in body temperature.

Defensive behaviors of rats have also been examined in a visible burrow system, following the presentation of a cat (Blanchard and Blanchard, 1989). The defensive behaviors are examined in great detail as a function of time after the removal of the cat. Withdrawal, immobility, increased risk assessment, and a suppression of nondefensive behaviors such as eating, drinking, grooming, and mounting are quantified. This paradigm has a high degree of face validity as a method to assess fear and anxiety.

Innate Anxiety-Like Behavior in Animals Other Than Rodents

Observer-rating methods have been used to assess anxiety in primates. The observation of struggling in a restraining chair, behaviors such as head- and body- turning, periods of immobility, scratching, and vocalization may be behavioral manifestations of anxiety (Ninan, 1982). In addition, behavioral as well as physiological assessment of the hypothalamic-pituitary-adrenal (HPA) axis has been carried out in monkeys separated from their social groups (Mendoza et al., 1978). However, to assess a direct relationship between the model and anxiety, these behaviors should be selectively elicited by other anxiety-provoking stimuli, and not observed in nonanxious animals. There is often an assumption that behavioral studies in non-human primates do not require the same degree of validation as those using other species. While the phylogenetic closeness to humans makes studies in primates extremely valuable, such studies should be as rigorously controlled as studies in other species.

In recent years, the zebrafish *Danio rerio* has emerged as a vertebrate model organism suitable for unbiased genetic analysis (Driever et al., 1996; Haffter et al., 1996) and large-scale small molecule screening (Zon and Peterson, 2005). As a model for anxiety, zebrafish is still at its infant stage (Guo, 2004). Despite that, several behavioral paradigms have been exploited, which include light-dark transition (Guo, 2004), behavioral responses to alarm substance (Speedie and Gerlai, 2007), anti-predator behavior (Bass and Gerlai, 2008), and novelty-elicited diving response (Levin et al., 2007). Rigorous pharmacological validations of these behavioral paradigms remain to be carried out.

9.3.1.2 Assessing Learned Anxiety-Like Behaviors

When a neutral stimulus such as a tone (called conditioned stimulus) is paired with an aversive stimulus such as a foot shock, the tone can now elicit a constellation of

behaviors that are typically used to define a state of fear in animals. Since these behavioral responses are similar in many respects to the constellation of behaviors that are used to diagnose generalized anxiety in humans, assessing this type of conditioned fear yields insights into human anxiety.

The Conditioned Emotional Response

In the prototypic experiment, food-deprived rats are first trained to press a lever for food using intermittent reinforcement. After giving a sufficient number of training sessions to establish stable rates of bar pressing, a neutral stimulus such as a light or a tone, is paired with a shock. After a small number of pairings, the neutral stimulus alone produces a suppression of the lever-pressing behavior (Estes and Skinner, 1941). Although such conditioned suppression is generally viewed as a reflection of a central state of fear, it is clear that a suppression of lever pressing alone is not sufficient to infer a central state of fear. Salient or novel stimuli by themselves can suppress bar pressing. Thus, in addition to suppression of bar pressing, assessment of other phenotypes, which are often observed in conditioned suppression, such as defecation, urination, fast and shallow breathing, and freezing, will further strengthen the link to fear or anxiety. Moreover, drugs that are known to reduce fear or anxiety should also attenuate the conditioned emotional response. Indeed, many drugs have been tested using this paradigm. In general, there has been success in differentiating between drugs that are anxiolytic and non-anxiolytic in humans. For example, benzodiazepine, barbiturates, and opiates generally attenuate the conditioned emotional response, whereas chlorpromazine and amphetamine do not. However, exceptions to this generalization can be found for every drug class, and this assay is in general viewed as too variable to be used for screening novel anxiolytics.

A simpler paradigm than the conditioned suppression of bar pressing is the following: a rat is given a tone (CS) followed by an electric shock (US). After a few tone-shock pairings, defensive responses including freezing, changes in heart rate, blood pressure, hormone release, and pain sensitivity will be elicited by the tone. This simple paradigm of fear conditioning has been used to dissect the neural circuits involved (see later sections).

The Fear Potentiated Startle

The amplitude of the acoustic startle reflex in the rat can be augmented by the presence of a cue (e.g., a light) that has been previously paired with a shock (Brown et al., 1951). As it is common sense that people startle more when they are afraid, this “fear-potentiated startle” has good face validity as a model for anxiety. Indeed, it has been replicated using either an auditory or a visual conditioned stimulus, when startle has been elicited by either a loud sound or an airpuff (Davis, 1986).

Fear-potentiated startle offers a number of advantages for analyzing how drugs affect fear or anxiety. Perhaps the most important advantage is that it is defined as a within-subject difference in startle amplitude in the presence or the absence of the conditioned stimulus, thus making it a sensitive measure, by reducing problems caused by between-subject variability in startle. Indeed, a variety of drugs that reduce fear or anxiety in humans decrease potentiated startle in rats. Drugs like clonidine, morphine, diazepam and buspirone, which differ considerably in their mechanism of action, all block potentiated startle. In most cases, these treatments do not depress startle levels on the noise-alone trials, although drugs like clonidine do have marked depressant effects on both types of trials. In addition, drugs like yohimbine and piperoxane, which are anxiogenic in humans, also increase potentiated startle in rats.

9.3.2 Models for Other Anxiety Disorders

9.3.2.1 Animal Models for Phobic Disorders

A phobia is a fear of a situation that is out of proportion to its danger, can neither be explained or reasoned away, is largely beyond voluntary control, and leads to avoidance of the feared situation. It appears that phobias develop more readily to some stimuli (such as spiders and snakes) than to other dangerous objects. In addition, phobias can be transmitted by observation in animals. For example, Rhesus monkeys raised in captivity do not have a fear of snakes, but rapidly acquire such a fear by watching an adult monkey behaving fearfully in the presence of a snake. Interestingly, the fear of a flower cannot be transmitted in a similar manner (Cook et al., 1985). Some phobias in animals appear to be innate. For example, specific fears of poisonous coral snakes are found in some snake-eating birds that have never seen such a snake before (Smith, 1975, 1977).

Although there has been much research on animal phobias from the ethological and psychological points of view, there is very little pharmacological data from animal studies that can be considered directly relevant to the treatment of human phobic disorders. In humans, behavioral therapy, i.e., exposing patients to their phobic stimuli remains the most efficacious treatment for phobic disorders. Future pharmacological research using animal models will be used in conjunction with the behavioral therapies to benefit patients.

9.3.2.2 Animal Models for Obsessive Compulsive Disorder (OCD)

OCD is an anxiety disorder that afflicts 2–3% of the population with recurrent intrusive thoughts and ritualized actions, causing significant impairment of normal lives. Serotonin reuptake inhibitors (SSRI) are the only effective pharmacological treatment for OCD.

Based on behavioral similarity, naturally occurring repetitive or stereotypic behaviors observed in animals have been examined. These include tail chasing, fur chewing, and weaving, innate motor behaviors that occur during conflict, frustration or stress (displacement behaviors) such as grooming, cleaning and pecking. The effect of SSRIs has been tested in some models (Altemus et al., 1996; Nurnberg et al., 1997). Although some of these models have good predictive validity in addition to face validity, many have not been used since the original publications. To date, three behavioral models are in use: The barbering (Garner et al., 2004), marble burying (Treit, 1985), and signal attenuation models (Joel and Avisar, 2001). For instance, barbering is a common behavior in laboratory mice, where barbers pluck hair from their companions. Because not all mice perform such behavior, it has been viewed as an abnormal form of behavior, which shows similarity to compulsive hair plucking (trichotillomania) in humans (Garner et al., 2004). Although barbering seems to have strong face validity as a model of trichotillomania, it currently lacks pharmacological validation. Barbering has an important advantage over other models, in that it develops spontaneously, and thus may provide insights into the etiology of trichotillomania.

9.4 Neural Circuitry Underlying Anxiety-Like Behaviors in Animal Models

The neural circuitry underlying learned anxiety-like behavior has been studied in the context of the fear-conditioning paradigm. Conditioned fear is mediated by the transmission of information about the CS and US to the amygdala, which then sends output projections to the behavioral, autonomic, and endocrine response control systems located in the brainstem (Davis, 1992; Fanselow, 1994; Kapp et al., 1992; LeDoux, 1992). Pathway tracing and lesion studies suggest that lateral amygdala (LA) is the sensory gateway to the amygdala, and therefore the first possible site in the amygdala where cells processing the CS might be modified by association with the US in fear conditioning. With the basic elements of the circuitry understood from lesion studies, fear plasticity in the amygdala has been studied in the following ways: First, single-unit recording; second, LTP; third, drugs that block LTP are infused into amygdala areas where LTP is believed to occur, and effects on the acquisition of conditioned fear behavior assessed.

The neural circuitry underlying innate anxiety-like behaviors is also thought to involve the amygdala as an important structure: threatening stimuli perceived by the sensory system are transduced to the sensory thalamus and sensory cortex. The lateral nucleus of the amygdala receives input signals from the thalamus, the hippocampus, and the cortex, and projects to the central nucleus of the amygdala, which in turn extensively projects to the hypothalamus, midbrain, and brain stem areas. Indeed, lesioning of the amygdala in rats completely abolishes rats' innate fear defense response toward a cat (Blanchard and Blanchard, 1972; Misslin and Ropartz, 1981).

9.5 Molecular Genetic Understanding of Anxiety in Animal Models

The availability of gene targeting and transgenic technology in mice has led to the finding of many knockout and transgenic animals that exhibit abnormal anxiety-like behaviors. Some of the identified genes fit into the neural systems that have been implicated in regulating anxiety, based on the study of neural circuitry, whereas others seem to be required for general neural functions, implying that the overall well-being of the nervous system is critical to maintain a normal level of anxiety.

Enhancement of the GABA neurotransmitter system function is one of the effective therapeutic treatments for anxiety disorders. Targeted inactivation of GABA synthesis enzymes or certain GABA receptors indeed lead to increased anxiety-like behaviors (Kash et al., 1999; Löw et al., 2000).

Deregulation of the serotoninergic system has also been strongly implicated in anxiety and depression. Targeted inactivation of the 5-HT_{1A} receptor gene leads to increased anxiety-like behavior in mice (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). Interestingly, through a tissue specific conditional rescue strategy, it is shown that the expression of 5-HT1A in the hippocampus and cortex could rescue the increased anxiety phenotype in the 5-HT1A mutant. Treating the "rescued" mice with doxycycline to shut off 5-HT1A expression during development or during adulthood shows that receptor expression before day 21 is crucial for normal anxiety-like behavior (Gross et al., 2002).

Like SSRIs, noradrenaline reuptake inhibitors are also effective in treating depression. In addition, inhibitors for the monoamine oxidase A (MAO-A), which block the metabolism of noradrenaline, serotonin, and dopamine, are used in the treatment of anxiety disorders. Mutant mice lacking the MAO-A gene have a reduced anxiety-like phenotype (Cases et al., 1995). However, additional studies suggest that a more general alteration in sensory ability or selective alteration in emotional behavior might have contributed to the observed differences (Kim et al., 1997).

Corticotrophin-releasing hormone (CRH) is the primary regulator of HPA axis. Stress induces the release of CRH from the hypothalamus, which causes the release of adrenocorticotropin (ACTH) from the pituitary into the general circulation. ACTH then induces the release of glucocorticocoid stress hormones from the adrenal glands (Miller and O'Callaghan, 2002). CRH activity is mediated by two main receptors, CRH-R1 and CRH-R2 (Eckart et al., 2002). Both receptors also bind urocortin, a neuropeptide related to CRH (Vaughan et al., 1995). Deletion of the CRH gene in mice significantly decreased basal corticosterone (CORT) levels and markedly blunted the stress-induced increase of CORT levels in the circulation. However, at the behavioral level, the CRH mutant mice have a normal anxiety-like phenotype (Dunn and Swiergiel, 1999), suggesting that either CRH is not involved in regulating anxiety-like behavior, or there is a genetic redundancy in the regulation of anxiety-like behavior. Since the CRH transgenic mice have a heightened level of anxiety (Stenzel-poore et al., 1994), this finding suggests that redundancy is likely to be the explanation for the lack of behavioral phenotype in CRH mutant

mice. CRH-R1 knockout mice show reduced levels of anxiety (Smith et al., 1998; Timpl et al., 1998). However, the story with CRH-R2 knockout mice is more complicated, as results differ among the three groups that have independently inactivated CRH-R2 (Bale et al., 2000; Coste et al., 2000; Kishimoto et al., 2000). CRH-R2 knockout mice are reported to have either no difference in anxiety-like behaviors or have a heightened anxiety-like phenotype. Discrepancy regarding the role of CRH-R2 could be either due to differences in the procedures, or in the genetic background. Mice deficient in both CRH receptors have an impaired HPA response to stress and a sexual dichotomy in anxiety-related behaviors (Bale et al., 2002; Preil et al., 2001).

9.6 The Role of Environment

In addition to many genetic factors involved in regulating anxiety as described above, human experience suggests that the environment can have a strong influence on an individual's susceptibility to anxiety disorders. Studies using animal models show that maternal behaviors can have an influence on the development of strain-specific difference in anxiety-like behavior in mice (Francis et al., 2003). Thus, future studies of how environmental factors interact with genetic components to affect neural circuitry functions will provide further insights into anxiety and anxiety disorders.

9.7 Summary and Perspectives

Various anxiety disorders manifest in humans. For most of them, effective and safe treatments are not yet available. Studies of fear or anxiety in animal models have led to various behavioral assays, which have been or can be used for pharmaceutical screening of small molecule drugs that may prove useful in the treatment of human anxiety disorders. As discussed in this chapter, the validity of various animal models needs to be carefully evaluated before using them to screen for drugs that are targeted to specific human anxiety disorders. Secondly, the assays should have a high enough throughput so that a large number of diverse chemicals can be screened with relative ease. Because of the complexity and diversity of the chemical space, large-scale screens are necessary to identify novel small molecules to be further evaluated down the pharmaceutical pipeline. In this regard, the newly emerged model organism, the zebrafish *Danio rerio* may prove advantageous for the first round of large-scale chemical screening.

In addition to serving as a platform for drug screening, animal models of anxiety are critical to our fundamental understanding of the molecular and neural mechanisms underlying the regulation of fear or anxiety. Such improvement in our fundamental understanding is important for rationalized drug discovery. Regarding the

neural circuitry of fear or anxiety, we have learned that the amygdala is a central component of the circuitry. In the future, it would be important to determine how the "emotional" value of certain sensory stimuli is encoded by the sensory system and conveyed to the amygdala, and how the amygdala further regulates behavioral output. Although gene-targeting studies in mice have led to a vast array of genes, which is involved in anxiety-like behaviors, our knowledge of how genes and environment regulate the development and function of the anxiety circuitry remains rudimentary. Future work using animal models, building upon the current foundation, promises to further uncover the molecular and neural basis of anxiety and anxiety disorders, and lead to effective treatments of these disorders.

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