Chapter 9

Genetic Animal Models of Anxiety

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Abstract

Conditions such as generalized anxiety disorder, panic disorder, social phobia, specific phobia, obsessive-compulsive disorder, and post-traumatic stress disorder cause undue suffering and economic burden on a substantial portion of our society. The prevalence and serious debilitating effects of anxiety disorders increases the necessity for fast and efficacious understanding of the neurobiological pathways associated with these maladies. While the neural underpinnings of this spectrum may have been identified, further analysis is necessary to generate pharmacologically significant data. The development of new molecular genetics techniques applied towards the generation of specific knockout models with anxiety-like phenotypes have been instrumental to our understanding of anxiety spectrum disorders due to their specificity of effected targets. This chapter will discuss the individual anxiety spectrum disorders with a focus on the animal models displaying relevant phenotypes for neurobehavioral research.

Key words: Anxiety, Anxiety spectrum disorders, Anmal models, Exploration, Fear

1. Introduction: Anxiety Spectrum Disorders

The physiological and behavioral changes induced by anxiety represent a natural adaptive response to danger or conflict (1). However, when anxiety lasts for more than six months in the absence of any actual danger and begins to disrupt the ability to cope with normal daily activities, the condition is considered pathological (1, 2).

Anxiety disorders have emerged as the most prevalent type of mental illness in the United States (3, 4), affecting approximately 18% of Americans each year (2). The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) groups several disorders together under the of anxiety spectrum, including panic disorder, agoraphobia, generalized anxiety disorder (GAD), social phobia,

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specific phobia, post-traumatic stress disorder, and obsessive-compulsive disorder (OCD) (3, 4). However, the nature of the distinctions between each of these specific disorders is such that significant comorbidity is common (3, 4). In addition, anxiety spectrum disorders are likely to be comorbid with other mental illnesses (2), such as substance abuse or depression (3, 4).

GAD is characterized by excessive worry on a daily basis lasting six months or longer (2). It is a distinct disorder, despite its frequent comorbidity with other anxiety spectrum disorders and major depressive disorder (5, 6). While GAD sufferers generally recognize the unnecessary intensity of their anxiety, the exaggerated worry is difficult to assuage. Physical symptoms of the disorder include: fatigue, headache, muscle tension, muscle aches, difficulty swallowing, trembling, twitching, irritability, sweating, nausea, lightheadedness, having to go to the bathroom frequently, shortness of breath, hot flashes, and sleep complaints (2–4). As only one half of diagnosed individuals report full-time employment, and 40% receive public assistance, the disability associated with GAD is robust (3, 4).

The central symptom of panic disorder is the presence of attacks of intense fear or anxiety, usually abrupt and rapid in occurrence (3, 4). A panic attack may occur with or without a situational trigger, and, according to the DSM-IV, it includes at least four of the following symptoms: dyspnea (respiratory distress), choking sensation, heart palpitations, chest pain, sweating, trembling, numbness or tingling sensation, nausea, chills, hot flashes, vertigo, derealization, and fear of dying, losing control, or "going crazy" (3, 4). While panic attacks may accompany many other mental disorders, the key feature of panic disorder is repeated occurrence of spontaneous attacks, i.e. those with no situational trigger. Additional symptoms of panic disorder include anticipatory anxiety of the next attack and avoidance of situations thought to trigger the attacks that can lead to agoraphobia (3, 4).

Post-traumatic stress disorder (PTSD) occurs after a person witnesses or experiences an event involving harm to themself or others, such as a rape, plane crash, natural disaster, or war experience (2, 7). While some anxiety is to be expected after a stressful event, in order to be classified as PTSD, a patient must exhibit the following symptoms for at least one month: persistent re-experiencing of the event in dreams, recollections or flashbacks; avoidance of stimuli that trigger memory of the event, emotional numbing, or feelings of detachment; hyperarousal indicated by hypervigilance, irritability, anger, impaired concentration, difficulty sleeping, or exaggerated startle response (3, 4, 7). Lifetime prevalence of PTSD is estimated at between 1 and 9% (3, 4), and is four times more prevalent in women than in men (7). PTSD is often comorbid with other disorders, especially other anxiety disorders, depression, and substance abuse (2–4, 7), and increases the

chances of suffering from other health risks such as obesity, diabetes mellitus, heart disease, arthritis (7), peptic ulcers, and hypertension (3, 4).

Social anxiety disorder, or social phobia, is a distinct fear of being judged or embarrassed in social or performance situations, followed by avoidance of these situations (3, 4, 8). In severe cases, nearly all interpersonal interactions evoke intense anxiety, while mild cases may involve a fear of public speaking or meeting strangers (3, 4). Behavioral and physiological symptoms of social anxiety disorder may include: blushing, twitching, stammering, avoiding eye contact, palpitations, sweating, gastrointestinal discomfort, and muscle tension (3, 4). In children and adolescents, social anxiety disorder may manifest as clinging, crying, school refusal, vague somatic complaints, stuttering, fidgeting, test-anxiety, behavioral problems (e.g. fighting, stealing, or truancy) and mutism, in addition to typical adult symptoms (3, 4).

An intense and irrational fear of something that poses little or no threat is referred to as a specific phobia (2). Despite the acknowledgement that these fears are irrational, adults with specific phobias often experience severe anxiety or panic when they are faced with, or thinking of, the object or situation. There are many subtypes of specific phobias. Commonly, the patient demonstrates a phobia of the natural environment (such as storms, water or heights) and/or certain alarming circumstances (such as enclosed areas or crowded elevators). Others will have an aversion to specific animal species (such as spiders) or harmful scenarios (such as where there is a risk of coming into contact with blood). In certain cases, avoidance of specific phobias can become disabling.

Approximately 3% of the population is affected by OCD, a condition characterized by uncontrollable repetitive thoughts which lead to ritual behaviors that reduce the anxiety generated by those thoughts (9). Performing rituals does not provide pleasure to patients (2), and adults with the disorder are often aware that their behavior is irrational (10). The disorder affects males and females with the same incidence and the symptoms most commonly appear at any time from childhood to early adulthood. Genetic studies suggest that genetic factors play a significant role in the pathogenesis of OCD (11). For more details on clinical aspects and animal models of obsessive-compulsive spectrum disorders, see the chapter on OCD in this volume.

2. Genetic Factors and Neural Underpinnings

Research has established a clear genetic basis for anxiety-related behaviors. Sensitivity to stress or threat may be a critical factor in predisposing individuals to anxiety spectrum disorders (1).

In humans, studies show that first-degree relatives of anxiety disorder patients are considerably more likely to develop anxiety disorders than controls subjects (19.5% of GAD patient first-degree relatives, compared to 3.5% of control subjects) (3, 4). Furthermore, targeted gene mutations in mice have revealed that the modification of gene expression has a significant effect on anxiety phenotypes (1).

It has been proposed that in states of fear or arousal, feelings of anxiety are organized in the locus ceruleus (LC), from which the ascending noradrenergic systems originates (1). Neurons in the LC may project to the paraventricular nucleus (PVN) in the hypothalamus and activate the hypothalamo-pituitary-adrenal (HPA) axis, which further triggers the anxious response. LC neurons may also project to other areas of the brain frequently involved in the anxiety response, such as the amygdala, prefrontal cortex, bed nucleus of the stria terminalis, hippocampus, pariaqueductal gray, hypothalamus, thalamus, and the nucleus tractus solitarius (1). Differences have also been found in the serotonergic, GABAergic, neurosteroid, and HPA axis hormone systems in patients with anxiety disorders (1).

The amygdala has been specifically targeted in anxiety spectrum disorder research due to its key role in regulating emotional behaviors (1). Previous studies implicate the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to physiological stress in rats (1). Additionally, both the amygdala and thalamic pathways may be responsible for primary appraisal of threat through automatic and fast analysis of harmful stimuli (1).

3. Experimental Models of Anxiety

The elevated-plus maze is a popular and reliable method of testing anxiety phenotype in laboratory animals. With two closed arms and two open arms, this paradigm pairs the innate rodent drive to explore with the fear of illuminated, open areas. Anxious rodents will spend less time in open arms than closed arms, display fewer exploratory behaviors (e.g. wall leans, vertical rears), and exhibit more stretch-attend postures or freezing. Anxiolytics have been shown to reduce these anxious phenotypes and increase exploratory behaviors (12).

Similar to the elevated-plus maze, the open field test measures general motor activity and exploratory behaviors in animals presented with the contradiction between exploring novelty and avoiding brightly lit areas. Anxious rodents placed in the open field test will display fewer exploratory behaviors and a significant increase in thigmotaxis, or spend more time in the periphery of

the open field than the center. After anxiolytic administration, rodent thigmotaxis will typically decrease and exploratory behaviors will become more prevalent (13).

Anxiogenic stimuli have been shown to increase digging behavior in rodents. Based on this information, the marble burying test can accurately represent an anxiety phenotype through the measurement of burying or digging behavior. Mice treated with non-sedative doses of anxiolytic benzodiazepines show a significant decrease in marble burying behavior, while their control counterparts buried roughly 75% of marbles present (14–16). While not a direct measurement of anxiety, the marble burying test is a pharmacologically sensitive paradigm, capable of assessing a natural, species-specific response to anxiogenic stimuli.

Grooming analysis is another valuable tool in the evaluation of rodent anxiety. The Grooming analysis algorithm, a combination of qualitative and quantitative measurements, provides a micro-behavioral assessment of a robust rodent activity. Anxious mice display disrupted grooming bouts, incorrect transitions, and a longer duration of grooming overall than control mice. After anxiolytic administration, these anxiety behaviors are reduced (17).

The Suok test simultaneously examines anxiety, vestibular, and neuro-muscular deficits. Using animal exploration as a means of measurement, this test combines a balancing instability, novelty, and the threat of heights. These factors cause anxious mice to exhibit freezing or more frequent falls, while anxiolytic administration reduces these phenotypes. The light-dark modification of this paradigm adds an additional stressor (i.e. illuminated areas) for further analysis (18).

Another paradigm capable of assessing anxiety in rodents, the hole-board test, measures directed exploration (i.e. head dipping), which is thought to be independent of locomotor activity. Whereas control mice will show exploratory behaviors, anxious mice will have a lower number of head dips. Also pharmacologically sensitive, rodent behavior in the hole-board test varies with the administration of anxiogenic or anxiolytic drugs (19).

4. Genetic Animal Models of Anxiety

Genetically modified animals are widely used as models for human brain pathogenesis, including anxiety and depression (20, 21). Since recent data has strongly linked clinical anxiety to genetic factors (22), researchers are striving to parallel anxiety-related loci in both animals and humans, in order to expand our understanding of the genetic basis of anxiety. Advances in molecular genetic techniques have allowed for much more specific manipulation of gene expression in animals. The development and refinement

of these molecular methods to modify genetic expression, combined with already established experimental models for assessing animal anxiety-related behaviors, can further elucidate our understanding of the genetic factors underlying anxiety.

As already mentioned, there are also many experimental models which possess high validity for assessing anxiety and depression-like behavior in animals. Through the combination of genetically modified animals with comprehensive bio-behavioral testing, researchers are beginning to garner a greater understanding of how specific genetic factors influence behavior, particularly in regards to a wide spectrum of anxiety-like phenotypes (23, 24). The power of a given genetic model is mediated by the specificity of the model, the depth of knowledge of secondary effects and the question being evaluated (22). The following section will provide examples of studies which have utilized genetic techniques to alter anxiety phenotypes and develop improved treatments for anxiety disorders.

5. Generation of Knockout Mice

While there are several knockout models for multiple serotonin receptors, the 5-HT1A receptor knockout has been particularly informative with respect to the study of anxiety (22). For example, these 5-HT1A knockout mice express a severe anxiety phenotype in the elevated plus maze and the open field tests of anxiety, highly consistent with the generally observed anxiolytic effects of 5-HT1A agonists (25).

The generation of a 5-HT1A receptor mutant mouse is accomplished through genetic and molecular techniques. One example consists of isolating an appropriate targeting vector for the receptor gene protein-coding region which corresponds to the 5-HT1A receptor. The process begins by localizing an 80 to 100-kb genomic fragment from the genomic library of a known mouse strain (e.g.,129/SVJ, C57BL/6J) by employing PCR primers which are generated to sequence within the intronless 5-HT1A receptor gene protein-coding region. A 1.5-kb pstI genomic fragment, including a portion of the protein-coding region, is then replaced by a neomycin resistant cassette (under the control of a phosphoglycerate kinase promoter). This targeted mutation is designed to produce a loss of function by truncating the 5-HT1A receptor protein at the third cytoplasmic loop. The neomycin resistance cassette is flanked by 1.7 kb of homologous genomic sequence at its 5′ end and by 6.9 kb of homologous genomic sequence at its 3′ end. The mutated fragment is then cloned into a phosphoglycerate kinase-thymidine kinase plasmid (PGK-TK) containing the herpes simplex virus thymidine

kinase gene, and driven by the phosphoglycerate kinase promoter in a Bluescript SK vector (26).

The next step in this process involves the generation of homologous recombinant clones. This involves taking a standard mouse line and electroporating their embryonic stem (ES) cells with a linearized targeting vector, created through the previously described molecular processes. Neomycin is applied in a positive/negative selection strategy to increase the number of targeted clones based on their induced drug resistance. ES colonies which survive drug selection are then screened for homologous recombination by using Southern blot analysis. To isolate the expected 5-HT1A receptor gene fragment, a genomic fragment corresponding to a region 5′ of the expected integration site can be used to probe genomic DNA (digested with BamHI). Wild-type and mutant alleles are indicated by specific kilo-base pair lengths, in this case by a 12.3-kb fragment for the wild-type and a 6.5-kb fragment for the 5-HT1A mutant (27).

Once the ES cells have been verified to contain the correct base pair sequence, which either inactivates the gene's ability to synthesize or causes synthesis of a non-functional protein, male chimera mice are produced by injection of the ES cells into blastocysts. The resulting male mice are then breed with females of a desired mouse linage. Germ-line transmission of the targeted mutation is then verified by Southern blot analysis of tail DNA. Heterozygotes are then mated with mice of a desired linage, and the resulting heterozygous animals are then crossed, producing mice of all three genotypes (homozygous mutant, heterozygous, and wild-type).

In an effort to determine which areas of the brain are involved in 5-HT1A mediated behavior, researchers have also developed a tissue-specific inducible rescue of the 5-HT1A knockout (28). This was accomplished by direct insertion of a cassette containing a promoter, responsive to the tetracycline-regulated transcriptional activator protein (tTA), into the 5′ leader sequence of the 5-HT1A receptor gene (22). This insertion erased expression of the receptor by its native promoter. The genetic line carrying the insertion was crossed with another transgenic line in which the tTA protein is expressed under the control of the αCaMKII promoter. The tTA protein then successfully induced expression of the 5-HT1A receptor in postsynaptic target tissues.

Notably, 5-HT1A receptor expression was restored in the hippocampus and cortex, but not in the serotonergic neurons of the dorsal raphe nucleus. Importantly, the restoration of the 5-HT1A receptor was accompanied by a return of normal anxiety-like behavior, demonstrating that the lack of postsynaptic receptors, rather than pre-synaptic receptors causes the lack of anxiety initially observed in the 5-HT1A knockout strain line (22). This study highlights the important role genetic animal models serve in

studying the neurobiology underlying anxiety. Further, the results obtained demonstrate how the advancement of molecular techniques, particularly the ability to increase tissue specificity, allow for the testing of very detailed hypothesis that would otherwise be impossible to study through the traditional pharmacological/neurobiological methods. However, these traditional tools, when used in conjunction with genetic techniques allow for better understanding of animal anxiety and may lead to improved treatment for human pathogenesis.

6. Examples of Altered Anxiety Phenotypes

While the mutant and transgenic mice developed for anxiety research exhibit behaviors classifiable on the anxiety spectrum, each specific model has subtle differences in their behavioral phenotype. This is due to the complex and interrelated nature of the brain. Behavioral analysis of homozygous 5-HT1A knockout mice suggests that they exhibit elevated anxiety and a marked antidepressant-like response in a rodent depression model (27). In the open field test, 5-HT1A mutants display a significant reduction in exploration of the central area, staying near the walls of the test box. Aversion to open spaces and a preference for enclosed areas are consistent with rodent expression of anxiety. Similar responses are observed in the elevated plus maze, a pharmacologically validated paradigm for the assessment of anxiety. 5-HT1A receptor mutants spend a reduced amount of time, and were less active in the open arms of the maze when compared to their wild-type littermates. In addition, 5-HT1A mutants exhibit increased avoidance of a novel object, diminished frequency of approach, and increased time spent in their nesting area. Reduction in exploratory behavior and an avoidance of novel objects corresponds to an anxiety-like state in rodents (27). The combination of these behavioral phenotypes suggests that 5-HT1A mutant mice do indeed exhibit heightened levels of anxiety. Neurobehavioral assessments, as described, as well as evaluation of the neural mechanisms underlying the behavioral phenotypes expressed by the 5-HT1A receptor mutant mice are fundamental to anxiety research in affective disorder pathogenesis, and the role of the serotonergic system.

The level of functionality of serotonin, an integral neurotransmitter, is dependent on its availability, either at the pre-synaptic or post-synaptic levels. The serotonin transporter (5-HTT or SERT) is a cell membrane protein that regulates serotonin signaling via reuptake mechanisms, and is a rate limiting factor in the availability of serotonin. The SERT knockout mutant mouse is another genetic model which has been invaluable to the study of

abnormal anxiety-related behavior (29, 30). These mice demonstrate a range of behavioral and neurophysiological abnormalities that resemble symptoms of mood and anxiety spectrum disorders (30). The literature has reported that these mice show reduced aggression (31), reduced locomotor activity (32), altered responses to antidepressants (31), reduced REM sleep (33), and exaggerated neuroendocrine and adrenomedullary responses to stress (34). In addition, SERT knockout mice also exhibit increased anxiety-like behavior, reduced exploratory locomotion and serotonin syndrome-like behavior (35). The complex phenotype expressed by these mice, as assessed through behavioral evaluations, social interaction observation, and pharmacological administration, has high translatability to the study of human psychiatric pathogenesis on the stress and anxiety spectrum.

7. Conclusion

The prevalence and disability associated with anxiety spectrum disorders requires thorough analysis of the genetic and neurobiological factors underlying these maladies. Animal models of anxiety offer great promise in future translational research in the field of biological psychiatry. Despite the complex nature and frequent comorbidity of anxiety disorders, specific endophenotypes and clinical symptoms can be targeted and reliably translated. The vast selection of experimental models of anxiety offers reliable and translatable methods of animal behavioral testing. Knockout or transgenic mouse models allow researchers to further investigate the intricate interactions of genetic and environmental factors associated with anxiety spectrum disorders. With refinements in molecular techniques, our ability to generate specific knockout models paralleling human psychiatric disorders improves markedly. As such, our capacity to model and potentially treat these disorders more effectively also increases. Furthermore, neurobiological pathways can also be isolated, more precisely, aiding in the creation of efficient and direct medications, which would reduce unpleasant or harmful side effects.

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