PART I

Anxiety and Stress Disorders
NEUROBIOLOGY OF ANXIETY

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1.1 INTRODUCTION

“Anxiety” is the subjective feeling of heightened tension and diffused uneasiness. It is a normal reaction to threatening situations and serves a physiological protective function in eliciting avoidance behaviors. The majority of individuals respond to anxiety-evoking environment appropriately but with some individual differences. The range of appropriate responses to threatening situations can best be described by individual differences in personality traits, in particular in emotional (in)stability/neuroticism [1–3]. Both genetic and environmental factors contribute to emotional (in)stability and to personality traits in general [4]. Approximately 5–10% of individuals display an exaggerated response to real or perceived threat or interpret ambiguous situations as threatening and can be classified as suffering from anxiety disorders [5]. It may be conceptualized that these individuals lie outside of the normal range of individual differences in emotional (in)stability [6]. Indeed, emotional instability and anxiety share common genetic factors.

Although a genetic contribution to emotional instability (neuroticism) and anxiety has long been known, it is only recently that multipoint linkage analysis identified chromosomal regions that may harbor candidate genes [7, 8]. Also, genetic polymorphisms in the serotonin (5-HT) transporter (5-HTT), 5-HT1A receptor, and brain-derived neurotrophic factor (BDNF) have recently been associated with neuroticism and anxiety conditions [9–11]. The slow pace of discovering susceptibility genes in human is largely due to the complex genetics of personality traits and common disorders; thus, individual genes have a relatively small contribution to traits/diseases. The effect of the environment on the expression of anxiety disorders also complicates the elucidation of the underlying pathogenic processes.

Animal models have long been used in the research of anxiety. Quantitative trait locus (QTL) analysis suggests that the genetic basis of “emotionality”/fear reaction in mice can be defined as the variance of a set of few behavioral measures [12–15], such as avoidance of novel environment (abbreviated in this review as Av), behavioral inhibition/activity in highly or moderately threatening situations (Ac) and autonomic arousal (Aa). These dimensions of rodent behavior are reminiscent of the characteristics of emotional instability/neuroticism in humans. In the last decade, a large number of induced mutations have been generated by homologous recombination in the mouse, and some of these strains show a significant deviation from their parental strain in measures of fear response. The abnormal fear response of these mice can be conceptualized as anxiety-like, similar to anxiety disorders in humans. By analyzing a large number of these strains and by classifying them according to the three fundamental dimensions of noncognitive behavior proposed above (AvAcAa), it is possible to implicate multiple neurobiological processes in anxiety-like behavior. Furthermore, these genetic models allow the study of the combined effect of two or more genes as well as the interaction of genes and environment in the expression of anxiety-like behavior in mice. These results may be extrapolated to humans and they eventually could help to better understand the polygenic and multifactorial nature of human anxiety disorders.

1.2 PSYCHOLOGICAL TRAITS AND THEIR GENETIC BASIS

Since anxiety is the continuous expression of normal human personality traits, it is important to briefly summarize a few of the leading personality theories. Personality
Traits are underlying characteristics of an individual that can explain the major dimensions of human behavior. Traits are dimensions representing a continuum of characters and most people fall in between the extremes. Personality traits have a wide individual variation, but they are relatively stable in individuals over time [16]. Cognitive/intellectual and noncognitive/affective/psychological traits are two fundamental domains of personality. Although the separation of the cognitive and noncognitive domains of personality may be practical, these variables interact and influence each other. Among the psychological traits two, extroversion versus introversion (E) and emotional stability versus instability or neuroticism (N), are probably the most important. An additional dimension is psychoticism (P) in the PEN model [1, 2] (Fig. 1.1). Autonomic arousal is an integral part of neuroticism and it is characterized by increased heart rate and blood pressure, cold hands, sweating, and muscular tension. A similar system based on the broad traits of neuroticism, extroversion, and openness is the NEO personality inventory (NEO-PI) [3]. Other models hypothesize the existence of more than three fundamental traits. The Big 5 (B5) model has three other dimensions in addition to emotional stability and extroversion [17–19]. The revised (R) NEO-PI also has five factors, and besides neuroticism, extroversion, and openness, consists of the factors of agreeableness and conscientiousness [20] (Fig. 1.1). NEO-PI-R is a self-report inventory with a high retest reliability, item validity, longitudinal stability, consistent correlations between self and observer ratings, and robust factor structure that has been validated in a variety of populations and cultures [3]. Gray [21] has modified Eysenck’s PEN model by rotating the dimensions of neuroticism and extroversion by 45°, resulting in two new dimensions: anxiety (N+, E-) and impulsivity (N+, E+). Gray’s work, however, has been done mostly on animals. Still another personality assessment is Cloninger’s biosocial model, which conceptualizes temperament as consisting of the four genetically and biochemically distinct traits of harm avoidance, reward
dependence, novelty seeking, and persistence [22] (Fig. 1.1). Harm avoidance is
correlated with NEO-PI-R neuroticism. Reward dependence is related to the anxiety/
neuroticism/extroversion traits of other classifications (Fig. 1.1). Novelty seeking is
also related to these traits and is similar to impulsivity in the Gray hypothesis. Each
of these broad dimensions of personality is comprised of a number of smaller traits
which are narrower in scope.

Using the techniques of quantitative behavioral genetics, it became clear that
roughly 40–60% of the variation in most personality traits has a genetic base. Broad
personality traits are under polygenic influence [4, 23]. Recently, genomewide linkage
studies have been performed by using the EPQ (Eysenck personality questionnaire)
[1, 2] to identify chromosomal regions associated with neuroticism. A two-point and
multipoint nonparametric regression identified 1q, 4q, 7p, 8p, 11q, 12q, and 13q [7],
while another similar study using multipoint, nonparametric allele sharing and
regression identified 1q, 3centr, 6q, 11q, and 12p [8], confirming some of the linkages
in the previous study.

1.3 EXTRAPOLATION OF PSYCHOLOGICAL TRAIT OF NEUROTICISM
TO MOUSE BEHAVIOR

1.3.1 Emotionality as Measure of Avoidance, Behavioral Inhibition/Activation, and
Autonomic Arousal in Animals

Behavioral studies with various rodent strains indicate that a set of a few behavioral
measures can describe “emotionality,” a behavior similar to the psychological traits
of emotional instability/neuroticism in humans [12–15]. To be able to analyze and
compare a large number of animal studies, we have selected throughout this review
three commonly used measures of emotionality: avoidance of novel environment,
activity/behavioral inhibition in highly or moderately threatening situations, and
autonomic arousal (Fig. 1.2). Here we refer to this triad of behavioral measures as
AvAcAa (avoidance, activity, and arousal).

1.3.2 Quantifying Emotionality in Animals

Attempts to measure emotionality and stress response in rodents have yielded a large
number of tests [24–28]. Ten years ago it was estimated that there were over 30 such
tests in use [29], and modifications of earlier tests have likely increased this number
since then. Initially, the development of these tests was facilitated by the need of
preclinical identification and characterization of anxiolytics. Indeed, these tests are
often referred to as anxiety tests, anxiety-related tests, or animal models of anxiety,
even if most of them actually measure the normal reaction of animals to novelty and
stress.

The animal models measure either unconditioned or conditioned fear/anxiety-like
behaviors. Another classification is based on more specific behaviors such as social
and defensive behavior. Table 1.1 provides a short list of the more commonly used
tests while more thorough reviews of the assays can be found elsewhere [28–32].
Unconditioned exploration tests measure the natural conflict experienced by animals
to either explore a novel environment for food, water, or social reward or avoid it due
to potential unknown dangers. Measurements of avoidant behaviors, such as decreased exploration of a particular region of the testing apparatus, compared to overall locomotor activity provide a quantifiable measure to assess the level of conflict in such novel environments. In a laboratory setting, animals are introduced into a novel and more or less fearful environment and their avoidance, behavioral inhibition/activity, and autonomic responses are measured. For example, the elevated-plus maze (EPM) [33] consists of a cross with opposing pairs of arms which are either open or enclosed and is elevated above the ground. The normal rodent behavior is to prefer the enclosed compartment of the maze, which is less aversive. During normal exploratory activity, however, the animal will enter the open arms. These entries into and the time spent in the open arms are counted and used to assess the level of avoidance, although additional, more complex behaviors can also be

Figure 1.2 Measures of “emotionality” in rodents. The human trait of neuroticism is extrapolated to the measures of emotionality in rodents: avoidance, activity, and autonomic arousal (AvAcAa). AvAcAa can be quantified in well-established behavioral models. Exploration of a low and a moderate to highly threatening environment provides measures of avoidance and activity, while physiological functions provide measurements of autonomic arousal. (See color insert.)

### TABLE 1.1 Commonly Used Animal Tests of Anxiety

<table>
<thead>
<tr>
<th>Conditioned tests</th>
<th>Punishment-Induced: Geller-Seifter conflict, Vogel punished drinking</th>
<th>Fear: fear-potentiated startle, contextual/cued fear conditioning, passive/active avoidance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unconditioned tests</td>
<td>Exploration: elevated-plus and zero mazes, open field, light-dark box</td>
<td>Social Interactions: maternal separation social competition</td>
</tr>
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</table>


The elevated-zero maze (EZM) is a modification of the EPM where the four arms have been replaced by a circular track separated into four quadrants of alternating open and enclosed regions. Avoidance can also be assessed in the center of a brightly lit open field [34]. Animals tend to stay and move around the periphery of the field, since the open area and bright illumination are aversive. The avoidance measured in an open field is assessed by the number of entries into and the time spent in the center of the open field or the path length in this area. An adaptation of this test is the light–dark crossing task [35], consisting of a two-compartment box in which one area is dark and the other is brightly lit. This test uses the animals’ natural tendency to prefer the dark and to avoid the brightly lit area. In this case, the number of crosses into and the time spent in the light compartment reflect the level of avoidance. Most studies compare the open arm/light compartment/center field activity/time as a percent of total activity/time. Also, exploration in the less aversive areas of the test apparatus (closed arm/dark compartment, periphery of the open field) is often regarded as an assessment of general activity levels, and it has been proposed that avoidance in the more stressful areas cannot be interpreted unless locomotor activity in low-stress areas is normal [36]. However, out-of-cage test environments are stressful even if they are moderately threatening. This notion is supported by the finding that a QTL has been linked to both the suppression of general locomotor activity and high-stress-area avoidance in a study involving a large number of mice [15]. Therefore, a number of laboratories, including ours, prefer to score overall locomotor activity separately (e.g., in activity boxes) as one measure of emotionality [37, 38]. The EPM, open field, and light–dark box tests are viewed as straightforward and relatively simple tests to conduct and as such are frequently used. However, more complex methods which highlight different aspects of avoidant behavior are available but are less commonly used. For example, in the social interaction test, behavior such as sniffing, grooming, mounting, and contact are monitored and used to infer changes in emotionality [39].

Conditioned conflict tests assess punishment-induced avoidance of a conditioned behavior. The Geller–Seifter test [40] is based on the conflict between completing an appetitive conditioned response that is unexpectedly paired with an unpleasant stimulus, such as the delivery of a mild electric shock. The Vogel punished drinking test [41] is similar to the Geller–Seifter test but does not require an extensive training period for the conditioning of the measured response. In the Vogel punished drinking test, the subject is water deprived for 12–24 h and then placed into a testing apparatus containing a water bottle with a spigot from which the animal can drink. Thirsty subjects learn quickly that water is available from the spigot and will readily drink from it when repeatedly placed into the testing apparatus. During the test session, however, the water spigot is connected to an electrical source that provides a mild electric shock upon contact with the spigot, placing the animal in conflict of choosing the appetitive reward or avoiding it. The level of avoidance reflects the emotionality of the subject.

Emotionality can also be measured in conditioned fear paradigms [42] such as fear-potentiated startle and contextual fear conditioning. These tests involve the element of emotional learning, as a neutral stimulus such as sound or light is paired with an electric foot shock. After a few trials, the previously neutral stimulus becomes aversive when presented alone. Mobility and freezing time can be used as indices of behavioral inhibition. A similar test is passive/active avoidance, a
one-pairing fear-induced avoidance assay. An animal is placed into a compartment and it has to either remain in that compartment to avoid a mild shock (passive avoidance) or go to another compartment (active avoidance, or escape-directed behavior) to avoid the aversive stimulus. Overall, conditioned tests provide less between-subject baseline variability than unconditioned response tests, but most conditioned assays require extensive training and the use of additional groups for controlling potential differences in learning and memory.

1.4 ANXIETY: CONTINUOUS EXPRESSION OF NORMAL HUMAN PERSONALITY TRAITS

It has long been proposed that the underlying structure of normal adaptive traits and the maladaptive personality traits of anxiety are the same [22]. Analysis of normal personality traits by NEO-PI in persons with psychiatrist-ascertained anxiety disorders in a general population showed an association of high neuroticism with lifetime anxiety disorders [simple phobia, social phobia, agoraphobia, panic disorder, obsessive-compulsive disorder (OCD), and generalized anxiety disorder] (Fig. 1.1). Social phobia and agoraphobia were also associated with low extroversion, and OCD was associated with high openness to experience [43]. In the Cloninger model, anxiety incorporates many aspects of harm avoidance [22]. Autonomic arousal, an integral part of neuroticism, is also a characteristic of anxiety disorders and is manifested as tachycardia, increased blood pressure, and elevated core temperature [44].

Recent genetic studies further support the notion that anxiety is the continuous expression of certain personality traits. For example, neuroticism/harm avoidance share a common genetic variant with susceptibility to anxiety disorders. Lesch et al. demonstrated that a functional 5-HTT promoter polymorphism is associated with the NEO-PI-R factor neuroticism and harm avoidance of the Cloninger model [9]. Extension of these genetic studies to anxiety disorders by the same authors showed no differences in 5-HTT genotype distribution between anxiety patients and comparison subjects, but among anxiety patients, carriers of a specific 5-HTT allele exhibited higher neuroticism scores than noncarriers [45]. Over 20 other studies investigated this association, and recent meta-analyses of these studies found a small but significant association between 5-HTT polymorphism and in some but not all measures of neuroticism/anxiety [46]. These studies remind us of the multifactorial nature of anxiety and that individual genes have only a small contribution to the clinical phenotype.

1.4.1 Anxiety Disorders

In the United States, anxiety disorders are most often defined and diagnosed according to a categorical system established by the Diagnostic and Statistical Manual of Psychiatric Disorders, currently in its fourth edition (DSM-IV) [5]. The DSM-IV sets the boundary at which a particular level of emotionality becomes an anxiety disorder—a level often based on the number and duration of observable symptoms of anxiety. This categorical model of anxiety, although necessary for the clinical diagnosis of anxiety disorders, is far from being reflective of the biological
nature of emotional states. Emotionality and anxiety are more realistically illustrated through a dimensional model that encompasses a continuum of various measures. The subjectivity of diagnosis is further illustrated by the marked differences in the diagnostic criteria of generalized anxiety disorder between the DSM-IV [5] and the ICD-10 Classification of Mental and Behavioral Disorders [47]. Although a core group of symptoms is identical in the two systems, DSM-IV relates these symptoms to vigilance while ICD-10 emphasizes the importance of autonomic arousal/hyperactivity. For the purpose of the present review, a quick overview of the DSM-IV classifications of anxiety disorders will be presented, while further discussions on different diagnostic criteria of DSM-IV and ICD-10 can be found elsewhere [48, 49].

The DSM-IV provides diagnostic criteria for a number of anxiety disorders, including panic disorder, specific and social phobias, OCD, posttraumatic stress disorder (PTSD), and generalized anxiety disorder [5, 50]. Individuals suffering from panic disorder experience recurrent and unexpected panic attacks which lead to discrete periods of intense fear and/or discomfort. Panic attacks are characterized by increased autonomic responses, including increased heart and breathing rates, sweating, nausea, abdominal distress, chills or hot flashes, and lightheadedness. Panic disorder may include agoraphobia, defined as the avoidance of places or situations in which escape may be difficult or embarrassing in the event of a panic attack. Specific and social phobias are marked by persistent fear of either clearly discernible objects or situations or potentially embarrassing social or performance situations, respectively. Exposure to the phobic stimulus almost invariably leads to heightened anxiety that may be expressed as a panic attack. Phobic stimuli are most often actively avoided. OCD features recurrent obsessions and compulsions severe enough to interfere with everyday life. Obsessions are described as persistent and inappropriate anxiogenic ideas, thoughts, or impulses that are unrelated to a real-life problem. Individuals suffering from OCD reduce obsession-induced anxiety by performing repetitive behaviors known as compulsions. These excessive and stereotypic behaviors or mental acts are not realistically connected with what they are designed to neutralize (i.e., washing and cleaning, counting, checking, and rearranging, etc.). PTSD can develop following a traumatic event involving feelings of intense fear, helplessness, or horror (e.g., military combat, rape, assault, and serious accident). Patients experience distressing recollection of the event, numbing of general responsiveness, and persistent arousal. They make deliberate and persistent efforts to avoid trauma-associated stimuli. Finally, generalized anxiety disorder is characterized by persistent (over six months) and excessive worry, inability to control worry, muscle tension, irritability, and sleep disturbance that are not necessarily related to a specific threatening situation. Many individuals also experience somatic symptoms (dry mouth, sweating, nausea, urinary frequency) that are reminiscent of certain symptoms of panic attacks [5].

1.4.2 Anxiety-Like Behavior in Animals

Studying rodent behavior in various anxiety-related test paradigms (see Section 1.3.2) reveals variation in emotionality in these species [51–53]. This includes variability in avoidance of aversive environment (Av), activity/behavioral inhibition in highly or moderately threatening situations (Ac), and autonomic arousal (Aa)
Figure 1.3 The three dimensions (AvAcAa) of emotionality in rodents in three-dimensional representation. Avoidance (Av) is plotted on the x axis, the positive spectrum representing increased levels of avoidance in stress-inducing environments or following stress-inducing stimuli and the negative spectrum representing attenuation of avoidance or even increased risk-taking behavior. Activity (Ac) as a response to stress and fear is plotted on the y axis, with the positive spectrum corresponding to increased levels of activity in a moderate to highly stressful environment. Following habituation, the activity is not different. Decreased activity in such an environment would be plotted in the negative spectrum of the y axis. Finally, the z axis is used for autonomic arousal (Aa) elicited by fearful or stressful stimuli, including increased heart rate, increased blood pressure, heightened levels of muscle tension (as measured by the startle response), defecation, and urination. A positive or negative deviation from the normal level of autonomic arousal can be represented by the positive and negative spectra of this axis, respectively. The normal range of these measures in a population is represented by the grey cube. Increased anxiety-like behavior can be conceptualized as increased avoidance, reduced activity, and increased arousal beyond the normal range of variations, denoted by the red area of the cube. Reduced anxiety-like behavior or increased novelty-seeking/risk-taking behavior is characterized by attenuation of avoidance, increased activity, and reduced autonomic arousal, highlighted by the blue area of the cube. (See color insert.)

(Fig. 1.2). Figure 1.3 displays these three basic characteristics as dimensions that together determine the degree of emotionality (see gray box for the range of normal variation of the three dimensions).

Many selectively bred and genetically modified mouse/rat strains show significant deviations from the normal variability of these dimensions. Once measures of behavior in mutant rodents exceed the threshold of variance of the normal/control
population (increased avoidance, reduced activity, and increased autonomic arousal), the resulting condition can be conceptualized as anxiety-like and similar to anxiety disorders in humans (Fig. 1.3; see red area of the cube). Emotionality can also be decreased (attenuation of avoidance, increased activity, and attenuated autonomic arousal) in a novel environment (Fig. 1.3; see blue area of the cube). Indeed, individual animals with higher and lower emotionality have been selected from a population and bred selectively to obtain strains characterized with high and low anxiety-like behavior. The Maudsley reactive inbred rat strain shows a stable and reproducible deficit in exploratory behavior as compared to the Maudsley non-reactive strain [54]. A similar breeding strategy based on behavior in the EPM test (open arm entries and time) resulted in the high-anxiety-related behavior (HAB) and low-anxiety-related behavior (LAB) rat lines [55]. These differences in behavior presumably reflect contributions from multiple genetic loci. Since generating induced mutations in mice has become routine, numerous mutant strains with either increased or decreased anxiety-like phenotype have been identified (see detailed description of these lines in Section 1.8.2).

Mutant mice with increased emotionality/fear reactions can be used as models of anxiety, and it is important to determine if they have construct and face validity. The criterion of construct validity requires that the rationale used to form the animal model is based on the etiology and the biological factors of anxiety. Construct validity criteria are difficult to fulfill because factors underlying the human disorders are largely unknown. However, in a few cases, the animal model has a genetic defect similar to that identified in anxiety disorders. For example, reduced expression of the 5-HT$_{1A}$ receptor has been repeatedly shown in anxiety disorders and mice heterozygous for the inactivated 5-HT$_{1A}$ receptor have an increased anxiety-like phenotype (see Sections 1.6 and 1.10).

Face validity represents a similarity in the physiological and behavioral measures observed in humans and in the animal model. As with construct validity, some animal models meet this criterion more easily than others. Physiological expressions of fear as well as anticipation of fear are comparable across species as they include easily quantifiable autonomic or endocrine responses such as increases in heart rate, blood pressure, body temperature, and muscle tension or changes in plasma corticosterone.

Predictive validity refers to the sensitivity of the model to clinically effective pharmacotherapeutic drugs. Benzodiazepines, for example, are commonly used in the treatment of anxiety; hence, a proposed animal model with predictive validity should show decreased measures of anxiety following benzodiazepine administration. In contrast, anxiogenic compounds should produce the opposite in physiological and behavioral measures. In addition, compounds with no effect in the clinic should not alter these measures in an animal model. Although predictive validity is an essential criterion for an animal model of anxiety in preclinical research, it has less relevance in studies focusing on the pathogenesis of anxiety disorders. Indeed, sensitivity to anxiolytics such as benzodiazepines varies in the population [56, 57]. For example, it has been shown that subjects high in neuroticism [58] and panic disorder patients [59–61] are less sensitive to benzodiazepines, with true benzodiazepine treatment resistance occurring in up to 24% of panic patients [62]. A similar difference in drug response can also be seen in certain animal models of anxiety such as the 5-HT$_{1A}$ receptor–deficient mouse strains on various genetic backgrounds [38].
1.5 FEAR/ANXIETY CIRCUITS

1.5.1 Brain Regions Related to Anxiety Disorders

Anxiety is an emotion involving a complex interaction among many interconnected brain regions, with each component playing a specific role [63]. Most of these brain regions are part of the basic fear network, which is comprised of the prefrontal cortex, hippocampus, thalamus, and amygdala and its projections to brain regions responsible for coordinating the behavioral, autonomic, and endocrine response to fear (i.e., ventral tegmental area, locus ceruleus, dorsal motor nucleus of the vagus, nucleus ambiguus, lateral hypothalamus, paraventricular nucleus of the hypothalamus, etc.). Imaging technologies such as positron emission tomography (PET), magnetic resonance imaging (MRI), and functional MRI (fMRI) have made a large impact on elucidating the roles of various fear pathway structures in anxiety disorders. One of the best characterized limbic structures for its role in processing fear-related stimuli is the amygdala [64–66]. Furthermore, neuroimaging studies have shown that abnormal amygdala function is involved in anxiety disorders. Excess amygdala activation has been observed in PTSD patients in response to stimuli reminiscent of the traumatic event [67, 68] as well as in specific phobia patients when exposed to a phobia-related stimulus [69]. A volumetric MRI study revealed a significantly lower bilateral amygdala volume in panic disorder patients compared to individuals in the healthy control group [70]. Abnormal amygdala volume is not specific to panic disorder, as reduced amygdala volume has also been observed in patients suffering from OCD [71]. In contrast, larger right amygdala volume was measured in generalized anxiety disorder patients [72]. However, this particular study was performed in children; thus age, in addition to different anxiety diagnosis, may explain the contradicting results. Interestingly, the same cohort of children was later followed up with an fMRI study in which an exaggerated right amygdala response to fearful faces was observed in generalized anxiety disorder patients but not in healthy children. These results are suggestive of a relationship between structure and function and indicate that hyperactivity of the amygdala may be a characteristic feature of some anxiety disorders [73].

In addition to the hyperactivity of the amygdala, a number of neuroimaging studies have reported functional abnormalities in other fear pathway substrates in anxiety disorder patients. For example, increased levels of activity were found in the orbitofrontal cortex, hippocampus, and anterior and posterior cingulate in response to directed imagery of strongly emotional personal experiences in subjects suffering from panic disorder compared to healthy individuals [74]. Exaggerated activation of the orbitofrontal cortex has also been documented in specific phobia patients [69]. In contrast, the anterior cingulate gyrus showed lower levels of activity in PTSD during exposure to emotional stimuli by several groups [72, 75–77]. The anterior cingulate abnormality, together with the observed hyperactivity of the amygdala, has been incorporated into a neuroanatomical model of PTSD. Medial prefrontal structures, including the cingulate cortex, are thought to inhibit the activity of brain regions involved in fear responses, and therefore a hypoactive medial prefrontal cortex would fail to inhibit the amygdala in this model of PTSD [77, 78].

The hippocampus has also been extensively studied by neuroimaging techniques. Volumetric imaging studies that have been performed on PTSD patients have yielded
conflicting results with regard to hippocampal size. Some have found no differences in hippocampal volume between PTSD patients and non-trauma-exposed controls [79, 80], while others have documented either unilateral or bilateral reduction in hippocampal volume in PTSD patients [81–84]. A fundamental problem with most imaging studies is that the correlation between size of a neural substrate and the disorder in which it is documented may not be causal. In an attempt to address this issue, Wignall et al. [83] measured the hippocampal volume of recent-onset PTSD patients and found a decrease in right-sided hippocampal volume. Although the authors could not exclude the possibility that the hippocampal damage occurred during the time between the traumatic event and the onset of PTSD (mean of 158 days), they leaned toward the interpretation that smaller hippocampal volumes predispose individuals to the development of PTSD. A similar, yet longitudinal MRI study, however, showed that survivors of traumatic events who developed PTSD had no differences in hippocampal volumes at one week and at six months following the trauma when compared to trauma survivors that did not develop PTSD [80]. This particular controversy was somewhat abated by a study that found that monozygotic twins of PTSD combat veterans who themselves were not exposed to combat showed comparable hippocampal volumes to their combat-exposed brothers and that the hippocampi of these twins were significantly smaller than those of both combat veterans without PTSD and their non-combat-exposed twins [81]. These results indicate that a smaller hippocampal volume is a pre-existing PTSD predisposing factor rather than a product of the disorder. A reduced hippocampal volume is not, however, a prerequisite for the development of the disorder.

Certain dopaminergic substrates, particularly the ventral striatum, have been found to be both larger in volume [85] and functionally hyperactive in patients suffering from OCD. Other hyperactive regions documented include orbitofrontal cortex, caudate, thalamus, and the anterior cingulate cortex [85, 86]. Based on these data the prevailing hypothesis of OCD pathogenesis proposes that OCD symptoms are mediated in part by a defect in the orbitofrontal-subcortical circuits.

1.5.2 Brain Regions Related to Emotionality/Anxiety-Like Behavior in Animals

Brain regions involved in fear and emotionality in animals are largely the same as those implicated in anxiety disorders (Fig. 1.4). The amygdala has a central importance in the acquisition, retention, and expression of conditioned fear [87–89]. The amygdala seems to function as an emotional/cognitive interface receiving sensory information via projections from the cortex and the thalamus. Outputs from the amygdala to the frontal cortex are related to the conscious perception of fear while outputs to the locus ceruleus, hypothalamus, periaqueductal grey, and striatum mediate autonomic, neuroendocrine, and skeletal-motor responses associated with fear and anxiety.

Although it is widely accepted that the hippocampus plays an important role in certain forms of learning and memory, recent studies have show that the hippocampus is also involved in fear and emotionality. Interestingly, ventral hippocampal lesions affect anxiety while dorsal lesions result in defects in spatial learning. For example, cytotoxic lesions of the ventral hippocampus resulted in reduced aversion in the center of the open field, reduced freezing after footshock, and reduced inhibition in novelty-suppressed feeding [90]. Also, lesion of the ventral but not dorsal
hippocampus increased open-arm exploration in EPM [91, 92]. The septohippocampal system has been identified as being essential for the sensory processing of stimuli based on novelty and punishment [93]. Hippocampus has also been implicated in contextual fear conditioning [94].

Forebrain structures, including the medial prefrontal cortex (MPFC) and septum, are connected to the limbic system and their dysfunction has also been found in anxiety. Also, several studies have shown that lesions (cytotoxic and transection) of the MPFC inhibit fear-related behavior in rats [95–98]. These data indicate that MPFC promotes anxiety-like behavior. Finally, brain stem nuclei are important in the regulation of arousal. Of particular importance in anxiety are the noradrenergic locus ceruleus and the serotonergic raphe nuclei [99, 100].

1.6 NEUROTRANSMITTER SYSTEMS AND NEURONAL MESSENGERS IMPLICATED IN ANXIETY AND ANXIETY-LIKE BEHAVIOR

Traditionally, anxiety disorders have been viewed as disturbances in neurotransmitters, including γ-aminobutyric acid (GABA), 5-HT, norepinephrine (NE), dopamine...
(DA), and neuropeptides such as corticotropin-releasing hormone (CRH), cholecystokinin (CCK), and neuropeptide Y (NPY). Many of these neurotransmitters and their receptors have been identified as sites of action for anxiolytic drugs. However, neuronal messengers other than neurotransmitters such as cytokines have recently been implicated in anxiety. Here we summarize the relevant pharmacological data while a later section covers the pertinent genetic studies.

Alterations in GABA_A receptor function have long been implicated in anxiety disorders. For example, a deficit in GABA_A receptors has been identified in the hippocampus and parahippocampus of patients suffering from panic disorder and generalized anxiety disorder [101–103]. Furthermore, GABA_A receptor antagonists can elicit anxiety in patients with panic disorder, thereby mimicking a functional deficit of GABA_A receptors [104]. The GABA_A receptor is a pentameric ion channel typically composed of 2α(α1–6), 2β(β1–3), and 1γ(γ1–3) subunits [105, 106], and animal studies suggest that alterations in specific GABA_A receptor subunits are associated with certain forms of anxiety, such as withdrawal-induced anxiety [107, 108]. GABA_A receptor subunits have an especially important relevance in terms of the anxiolytic effect of benzodiazepines [109–111]. Classical benzodiazepines exert their effects by binding to multiple subtypes of GABA_A receptor, the predominant subtypes in the brain being those that contain α1,2,3,5 subunits. A recent report using receptor subtype–preferring compounds in nonhuman primate models concluded that α1 subunits containing receptors do not play a key role in the anxiolytic and muscle-relaxant properties of benzodiazepine-type drugs; instead, these effects involve α2,3,5 subunits containing GABA_A receptors [112]. Animal models have recently also been used to determine the GABA_A receptor subtype involved in the anxiolytic action of benzodiazepines (see description of these animals in Section 1.10).

Although lesion of 5-HT neurons in animals suggests a role for 5-HT in the control of anxiety states [113], the evidence for this notion is both conflicting and controversial. On the other hand, pharmacological manipulation of either the 5-HTT or the 5-HTT_1A receptor can clearly alter 5-HT neurotransmission and anxiety. The level of 5-HT is regulated by both the 5-HTT and the 5-HTT_1A autoreceptor (in the serotonergic raphe nuclei) [114, 115]. Inhibiting 5-HTT by selective serotonin reuptake inhibitors (SSRIs) has been shown to be very effective in certain anxiety disorders [114, 115]. Also, partial 5-HTT_1A receptor agonists such as buspirone have an anxiolytic effect [116]. Recently, animals with genetic modifications have significantly contributed to our understanding of the 5-HT system and the possible role of various 5-HT receptors in anxiety (see Section 1.10).

The role of NE in anxiety is based on its well-known involvement in stress reaction. Stress provokes and aggravates anxiety by increasing catecholamine release via the sympathoadrenal system in the periphery. In addition, NE neurons in the locus ceruleus play a critical role in the body’s response to alarm and threat. NE is believed to play an especially important role in anxiety disorders, such as panic disorder and PTSD [117, 118].

DA is mostly known as a mediator of reward and locomotor activity. However, these processes are also fundamental in personality traits and emotionality (Figs. 1.1–3), and the pharmacological manipulation of DA receptors have been reported to modulate anxiety-related behaviors. Specifically, agonists and
antagonists for the DA D₂ class of receptors (which includes D₂, D₃, and D₄ subtypes) have anxiogenic and anxiolytic properties, respectively [119–121].

A number of neuropeptides have been implicated in anxiety and have been suggested as therapeutic targets [122]. The stress response is mediated partly by the activation of CRH. CRH is produced in the hypothalamus (H), leading to the secretion of the adrenocorticotropin hormone (ACTH) from the pituitary (P), which in turn causes an increase in the synthesis and release of glucocorticoids from the adrenal glands (A) (HPA axis). The activation of the HPA axis is also involved in stress-related psychopathology such as anxiety disorders [123–125]. The maladaptive effects of chronic stress on the HPA axis have been extensively studied in both preclinical and clinical settings, and since a number of excellent reviews are available, this topic is not discussed further here [125–129]. In addition to the activation of the HPA axis and the consecutive release of the stress hormones, CRH is present outside of the hypothalamus where it is believed to participate in stress response [130]. Central administration of CRH in rodents produces behavioral effects that correlate with a state of anxiety such as reduced exploration in a novel environment or enhanced fear response [131–134]. Preclinical studies strongly implicate a role for central CRH, probably via the central noradrenergic systems, in the pathophysiology of certain anxiety disorders [125].

Glucocorticoids (corticosterone in rodents and cortisol in humans), the final effectors modulating the physiological response to stress, act via two receptor subtypes: the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) [135]. GRs are also the main regulators of a negative-feedback circuit that regulates the HPA axis following stress. Activation of GRs in the pituitary, hypothalamus, hippocampus, and frontal cortex decreases CRH gene expression, leading to a decrease in CRH release and the suppression of the stress-induced endocrine response [136, 137].

Another neuropeptide, NPY, has also been suggested to be involved in the clinical symptoms of anxiety [138]. In rats, central administration of NPY produces effects similar to that of anxiolytic drugs [139] whereas specific inhibition of the NPY-1 receptor by antisense oligonucleotide resulted in an increased anxiety-like behavior [140].

During the last few years, CCK has emerged as an important polypeptide in the central nervous system (CNS). There are several lines of evidence for a role of CCK in anxiety and panic attacks, and data also indicate that specific agonists to brain CCK(2) receptors produce anxiogenic-like effects while CCK(2) antagonists elicit anxiolytic-like responses [141–144].

Substance P has also been suggested to have a modulatory role in anxiety [145]. Substance P is released in response to aversive stimuli [146] and its administration in animal models elicits both anxiogenic and anxiolytic activity, depending on the dose and the specific brain region [122]. The receptor for substance P is the G-protein-coupled tachykinin NK-1 receptor which is expressed in brain areas associated with fear and anxiety [147]. Increasing numbers of reports indicate that specific antagonists of NK-1 receptors produce anxiolytic effects [148].

Although cytokines are not neurotransmitters and their primary role is in the immune system, several lines of evidence indicate that interleukin (IL) 1β, interleukin-6, and tumor necrosis factor (TNF) α modulate anxiety and mood [149].
Specifically, these proinflammatory cytokines elicit symptoms of anxiety/depression that may be attenuated by chronic antidepressant treatment. Also, immunotherapy using IL-2 or interferon (IFN) α, promotes depressive/anxiety symptoms [149]. Interestingly, the effects of cytokines are exacerbated by stressors, and chronic cytokine elevations may act synergistically with stressors [149].

1.7 GENETIC SUSCEPTIBILITY TO ANXIETY DISORDERS

A number of studies have sought to identify chromosomal regions and genes relevant to anxiety disorders. Although the results of linkage and association studies are inconsistent so far (see detailed description of these studies in [150–152]), candidate gene studies have yielded more consistent data. In several studies, a relatively small but significant increase in neuroticism was found in individuals who carry the s/s (short promoter repeat) alleles of the 5-HTT as compared to individuals with s/l (long) or l/l alleles [9, 153]. The s allele is associated with decreased transporter activity. Over 20 other studies extended this association to psychopathology, but not all found evidence for an association between 5-HTT polymorphism and anxiety [45]. However, recent meta-analyses of these studies found a moderate but significant association between 5-HTT polymorphism and NEO neuroticism [46] and TPQ (tridimensional personality questionnaire) harm avoidance [154]. The association of decreased transporter activity with anxiety is a rather surprising finding because pharmacological inhibition of the 5-HTT by SSRIs reproducibly results in an anxiolytic effect. However, the genetically determined reduction in 5-HTT activity in patients is present from early prenatal life and may affect brain development leading to anxiety in later life. Consistent with this notion, pharmacological inhibition of 5-HTT in early postnatal life in mice (which corresponds to late prenatal life in human) resulted in increased anxiety-like behavior in later life [155]. In summary, these data suggest that genetic or pharmacological reduction of transporter activity during brain development can lead to increased anxiety in adult life.

The 5-HT₁₅ receptor (5-HT₁₅R) has also been implicated in anxiety because reduced receptor levels were detected in the anterior cingulate, posterior cingulate, and raphe by positron tomography in patients with panic disorder [156]. These recent data complement previous reports that showed a deficit in the 5-HT₁₅R in PTSD and panic disorder patients [157–160]. However, no specific 5-HT₁₅R allele has been associated with anxiety disorders (a promoter polymorphism, on the other hand, has been linked to major depression and suicide [159]).

Although polymorphism in BDNF has primarily been studied in depressive disorder, the val allele of the Val66Met substitution polymorphism has recently been shown to be associated with higher mean neuroticism scores in the NEO- five factor inventory (NEO-FFI) in healthy subjects [161]. In another study the self-ratable state-trait anxiety inventory (Spielberger state-trait anxiety inventory) score, which allows anxiety to be quantified as a comparatively stable personality trait, showed a higher level of anxiety in Val/Val compared to Val/Met and Met/Met genotypes [11, 162]. These are surprising findings since it is the met allele that is hypofunctional (as a result of alterations in BDNF trafficking and secretion [163])
and because animal studies clearly show that genetic inactivation of BDNF results in anxiety (see Section 1.9).

1.8 GENETIC BASE OF ANXIETY-LIKE BEHAVIOR IN MICE

1.8.1 QTL Studies

QTL analysis of F2 hybrids of two strains of mice (A/J and C57BL/6J) that differ markedly in thigmotaxis and light-to-dark (LD) transition behaviors showed a linkage of LD to chromosome 10 (near D10Mit237; LOD of 9.3) and suggestive QTLs (LOD > 2.8) at chromosomes 6, 15, 19, and X [14]. In the open field, suggestive QTLs were mapped to chromosomes 6 and 14 [13]. These data indicate a lack of shared QTLs of fear/anxiety-associated behavior in various experimental paradigms (avoidance in LD and open field). Another group using multiple measures of avoidance and autonomic arousal found that the various measures are mapped to the same or nearby chromosomal location(s) [12, 15]. These studies used two relatively closely related mouse lines bred for differential anxiety-like behavior; thus a relatively small subset of genes may have changed during breeding. In contrast, the study that concluded a lack of shared QTLs in anxiety-like behavior [13] utilized the more distantly related A/J and C57BL/6J mice which presumably carried different anxiety-related alleles in multiple loci. This study may be easier to extrapolate to human populations characterized by a high degree of heterogeneity. So far, no genes have been identified in anxiety-related QTLs. Since QTLs are in the range of 10–30 cM, a region containing hundreds of genes, identification of linked genes within QTLs is difficult.

1.8.2 Anxiety-Like Behavior in Genetically Altered Mice

Recently, it has become possible to inactivate specific genes routinely in the mouse, and a large number of knockout strains have been generated. Many of the targeted genes have been implicated in anxiety, and the corresponding knockout strains have regularly showed behavioral abnormalities in anxiety-related tests (Tables 1.2 and 1.3). Beyond the mouse strains with inactivated “candidate” anxiety genes, anxiety-like behaviors were sometimes seen in mice with genetic inactivation in genes not obviously related to anxiety. These genes include intracellular signaling molecules and regulators of transcription/translation (Table 1.4). The association of these genes with anxiety-like phenotype indicates that anxiety is not limited to abnormalities of the neurotransmitter systems but can also be related to gene regulatory processes. Analysis of the genomic position of these genes shows that they are distributed throughout many chromosomes with no obvious clustering at any locus (Fig. 1.5).

One caveat of the analysis of anxiety-related knockout mouse strains is that the behavioral phenotypes are not always robust and are sometimes even questionable. Moreover, many variables can alter the interpretation of anxiety-related behavioral tests and tests are not standardized across laboratories and environmental factors. Furthermore, anxiety-like behavior may be part of a complex phenotype and secondary to major developmental or neuroanatomical defects. We limited our
### TABLE 1.2 Mice with Genetic Alteration of Neuronal Messengers Exhibiting Altered Anxiety Levels

<table>
<thead>
<tr>
<th>Targeted Gene/Protein</th>
<th>Behavioral Test</th>
<th>Avoidance (Av)</th>
<th>Activity (Ac)</th>
<th>Arousal (Aa)</th>
<th>Anxiety</th>
<th>Chr</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAD65 KO</td>
<td>OF, EZM</td>
<td>+</td>
<td>−</td>
<td>n/a</td>
<td>↑</td>
<td>chr2 qA3 (1)</td>
<td>[164]</td>
</tr>
<tr>
<td>COMT KO</td>
<td>L/D</td>
<td>+ (F only)</td>
<td>− (F only)</td>
<td>n/a</td>
<td>↑</td>
<td>chr16 qA3 (2)</td>
<td>[168]</td>
</tr>
<tr>
<td>NET KO</td>
<td>OF</td>
<td>n/a</td>
<td>−</td>
<td>n/a</td>
<td>?</td>
<td>chr8 qC5 (3)</td>
<td>[169]</td>
</tr>
<tr>
<td>5-HTT KO</td>
<td>OF, EZM</td>
<td>+</td>
<td>−</td>
<td>+ (?)</td>
<td>↑</td>
<td>chr11 qB5 (4)</td>
<td>[171]</td>
</tr>
<tr>
<td></td>
<td>OF, EPM, NSF, AA, FPS</td>
<td>+/−</td>
<td>0</td>
<td>n/a</td>
<td>↑/0</td>
<td>—</td>
<td>[172]</td>
</tr>
<tr>
<td>CRH overexpressing</td>
<td>OF, EPM</td>
<td>+</td>
<td>−</td>
<td>n/a</td>
<td>↑</td>
<td>chr3 qA2 (5)</td>
<td>[173, 174]</td>
</tr>
<tr>
<td>CRH KO</td>
<td>EPM, OF, and other</td>
<td>0</td>
<td>0</td>
<td>−</td>
<td>0</td>
<td>—</td>
<td>[175]</td>
</tr>
<tr>
<td>CRH-BP overexpressing</td>
<td>OF, EPM</td>
<td>−</td>
<td>+</td>
<td>0</td>
<td>↓</td>
<td>chr13 qD1 (6)</td>
<td>[180]</td>
</tr>
<tr>
<td>CRH-BP KO</td>
<td>EPM, OF and other</td>
<td>+</td>
<td>−</td>
<td>0</td>
<td>↑</td>
<td>—</td>
<td>[179]</td>
</tr>
<tr>
<td>NPY KO</td>
<td>OF, AS, EPM, PA</td>
<td>0</td>
<td>−</td>
<td>+</td>
<td>↑</td>
<td>chr6 qB2.3 (7)</td>
<td>[181]</td>
</tr>
<tr>
<td>ProEnkephalin KO</td>
<td>OF, EZM</td>
<td>+</td>
<td>−</td>
<td>n/a</td>
<td>↑</td>
<td>chr4 qA1 (8)</td>
<td>[182]</td>
</tr>
<tr>
<td>OFQ/N KO</td>
<td>OF, EPM, L/D</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>↑</td>
<td>chr14 qD1 (9)</td>
<td>[184]</td>
</tr>
<tr>
<td>BDNF cond KO</td>
<td>OF, EPM, L/D</td>
<td>+</td>
<td>n/a</td>
<td>+</td>
<td>↑</td>
<td>—</td>
<td>[185]</td>
</tr>
<tr>
<td>TNFα overexpressing</td>
<td>OF, L/D</td>
<td>+</td>
<td>+/−</td>
<td>n/a</td>
<td>↑</td>
<td>chr2 qE3 (10)</td>
<td>[190]</td>
</tr>
<tr>
<td>TNFα KO</td>
<td>L/D</td>
<td>+</td>
<td>−</td>
<td>n/a</td>
<td>↑</td>
<td>chr16 qA1 (11)</td>
<td>[192]</td>
</tr>
<tr>
<td>Interferon γ KO</td>
<td>OF, EPM</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>↑ (?)</td>
<td>—</td>
<td>[194]</td>
</tr>
<tr>
<td></td>
<td>OF, EPM, PA</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>↑</td>
<td>chr10 qD2 (12)</td>
<td>[195]</td>
</tr>
</tbody>
</table>

**Notes:** AvAcAa shown as decreased (−), increased (+), or not different (0) from wild-type (WT) controls. n/a = data not obtained by investigator. F = females; Chr = chromosome location and assigned number corresponding to illustration in Figure 1.5; OF = open-field exploration; KO = knockout; EZM = elevated-zero maze; L/D = light–dark box; EPM = elevated-plus maze; NSF = novelty-supressed feeding; AA = active avoidance; FPS = fear-potentiated startle; AS = acoustic startle; PA = passive avoidance; GAD65 = glutamic acid decarboxylase, 65-kD isoform; COMT = catechol-O-methyl transferase; NET = norepinephrine transporter; 5-HTT = serotonin transporter; CRH = corticotropin-releasing hormone; CRH-BP = CRH binding protein; NPY = neuropeptide Y; OFQ/N = orphanin FQ/nociceptin; BDNF = brain-derived neurotrophic factor; TNFα = tumor necrosis factor α.
<table>
<thead>
<tr>
<th>Targeted Gene/Protein</th>
<th>Behavioral Test</th>
<th>Avoidance (Av)</th>
<th>Activity (Ac)</th>
<th>Arousal (Aa)</th>
<th>Anxiety</th>
<th>Chr</th>
<th>Reference</th>
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<tbody>
<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;R&lt;sub&gt;\gamma_2&lt;/sub&gt; t&lt;sup&gt;+/−&lt;/sup&gt;</td>
<td>EPM, OF, L/D, PA, FC</td>
<td>+</td>
<td>−</td>
<td>n/a</td>
<td>↑</td>
<td>chr11 qA5 (13)</td>
<td>[196]</td>
</tr>
<tr>
<td>5HT&lt;sub&gt;1A&lt;/sub&gt;R KO</td>
<td>EPM, OF, EMZ and other</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>↑</td>
<td>chr13 qD1 (14)</td>
<td>[207–209]</td>
</tr>
<tr>
<td>5HT&lt;sub&gt;1B&lt;/sub&gt;R KO</td>
<td>OF, EPM, NSF</td>
<td>−</td>
<td>+</td>
<td>n/a</td>
<td>↓</td>
<td>chr9 (15)</td>
<td>[220]</td>
</tr>
<tr>
<td></td>
<td>EPM, FC, and other</td>
<td>0</td>
<td>0</td>
<td>n/a</td>
<td>0</td>
<td>—</td>
<td>[94]</td>
</tr>
<tr>
<td>CRH-R1 KO</td>
<td>L/D, EPM</td>
<td>−</td>
<td>0</td>
<td>−</td>
<td>↓</td>
<td>chr11 qE1 (16)</td>
<td>[224]</td>
</tr>
<tr>
<td>CRH-R2 KO</td>
<td>EPM, L/D, OF</td>
<td>+/0</td>
<td>0/+</td>
<td>+</td>
<td>↑</td>
<td>chr6 qB3 (17)</td>
<td>[225, 226]</td>
</tr>
<tr>
<td>DA D&lt;sub&gt;3&lt;/sub&gt; KO</td>
<td>OF, EPM</td>
<td>−</td>
<td>+</td>
<td>n/a</td>
<td>↓</td>
<td>chr16 qB4 (18)</td>
<td>[229]</td>
</tr>
<tr>
<td>α&lt;sub&gt;2A&lt;/sub&gt;-AR KO</td>
<td>EPM, OF</td>
<td>+</td>
<td>−</td>
<td>n/a</td>
<td>↑</td>
<td>chr19 qD2 (19)</td>
<td>[234]</td>
</tr>
<tr>
<td>Adenosine A&lt;sub&gt;2A&lt;/sub&gt; KO</td>
<td>EPM, L/D</td>
<td>+</td>
<td>−</td>
<td>n/a</td>
<td>↑</td>
<td>chr10 qC1 (20)</td>
<td>[236]</td>
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<tr>
<td>nAChR α&lt;sub&gt;7&lt;/sub&gt; KO</td>
<td>OF, L/D, AS, FC</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0/↓</td>
<td>chr7 qB5 (21)</td>
<td>[243]</td>
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<tr>
<td>nAChR α&lt;sub&gt;4&lt;/sub&gt; KO</td>
<td>EPM</td>
<td>+</td>
<td>−</td>
<td>n/a</td>
<td>↑</td>
<td>chr2 qH4 (22)</td>
<td>[244]</td>
</tr>
<tr>
<td>trkB overexpressing</td>
<td>EPM, L/D, FC</td>
<td>−</td>
<td>+</td>
<td>n/a</td>
<td>↓</td>
<td>chr13 qB2 (23)</td>
<td>[246]</td>
</tr>
<tr>
<td>NCAM KO</td>
<td>EPM, L/D</td>
<td>−</td>
<td>+</td>
<td>n/a</td>
<td>↓</td>
<td>chr9 qA5.3 (24)</td>
<td>[248]</td>
</tr>
<tr>
<td>L1 cond KO</td>
<td>EPM, OF</td>
<td>−</td>
<td>+</td>
<td>n/a</td>
<td>↓</td>
<td>chrX qA7.2 (25)</td>
<td>[249]</td>
</tr>
<tr>
<td>Cadherin 11 KO</td>
<td>EPM, AS, FC</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>↓</td>
<td>chr8 qD1 (26)</td>
<td>[250]</td>
</tr>
<tr>
<td>GIRK2 KO</td>
<td>EPM, L/D</td>
<td>−</td>
<td>+</td>
<td>n/a</td>
<td>↓</td>
<td>chr16qC4 (27)</td>
<td>[238]</td>
</tr>
</tbody>
</table>

**Notes:** AvAcAa shown as decreased (−), increased (+), or not different (0) from wild-type (WT) controls. n/a = data not obtained by investigator. Chr = chromosome location and assigned number corresponding to illustration in Figure 1.5; OF = open-field exploration; KO = knockout; EZM = elevated-zero maze; L/D = light–dark box; EPM = elevated-plus maze; FC = fear conditioning; AS = acoustic startle; PA = passive avoidance; GABA<sub>A</sub>R: γ<sub>2</sub> = γ-aminobutyric acid receptor A, γ<sub>2</sub> subunit; 5-HT<sub>1A</sub>/1B R = serotonin 1A or 1B receptor; CRH-R1/R2 = CRH receptor 1 or 2; DA D<sub>3</sub> = dopamine D<sub>3</sub> receptor; α<sub>2A</sub>-AR = α<sub>2A</sub>-adrenergic receptor; nAChR α<sub>7</sub>/4 = nicotinic acetylcholine receptor α<sub>7</sub> or α<sub>4</sub>; trkB = neurotrophin receptor tyrosine kinase B; NCAM = neural cell adhesion molecule; L1 = NCAM L1; GIRK2 = G-protein-coupled inwardly rectifying K<sup>+</sup> channel 2.
<table>
<thead>
<tr>
<th>Targeted Gene/Protein</th>
<th>Behavioral Test</th>
<th>Avoidance (Av)</th>
<th>Activity (Ac)</th>
<th>Arousal (Aa)</th>
<th>Anxiety</th>
<th>Chr</th>
<th>Reference</th>
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<tr>
<td>α-CaMKII KO</td>
<td>FC, OF</td>
<td>−</td>
<td>n/a</td>
<td>−</td>
<td>↓</td>
<td>chr18 qE1 (28)</td>
<td>[252]</td>
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<tr>
<td>PKCγ KO</td>
<td>EPM, L/D and other</td>
<td>−</td>
<td>+</td>
<td>n/a</td>
<td>↓</td>
<td>chr7  (29)</td>
<td>[255]</td>
</tr>
<tr>
<td>Fyn trk KO</td>
<td>L/D, PA</td>
<td>+</td>
<td>−</td>
<td>n/a</td>
<td>↑</td>
<td>chr10 qB1 (30)</td>
<td>[256]</td>
</tr>
<tr>
<td>NF-kB p50 KO</td>
<td>OF, EPM and other</td>
<td>−</td>
<td>+</td>
<td>n/a</td>
<td>↓</td>
<td>chr18 qG3 (31)</td>
<td>[260]</td>
</tr>
<tr>
<td>GR KO</td>
<td>L/D, EZM</td>
<td>−</td>
<td>+</td>
<td>0</td>
<td>↑</td>
<td>chr18 qB3 (32)</td>
<td>[263]</td>
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<tr>
<td>GR overexpressing</td>
<td>EPM, L/D</td>
<td>+</td>
<td>−</td>
<td>0</td>
<td>↑</td>
<td>—</td>
<td>[265]</td>
</tr>
<tr>
<td>VDR KO</td>
<td>OF, EPM, L/D</td>
<td>+</td>
<td>−</td>
<td>n/a</td>
<td>↑</td>
<td>chr15 qF4 (33)</td>
<td>[266]</td>
</tr>
<tr>
<td>CREB KO</td>
<td>OF, EZM, EPM, L/D</td>
<td>+</td>
<td>−</td>
<td>0</td>
<td>↑</td>
<td>chr1 qC2 (34)</td>
<td>[268]</td>
</tr>
<tr>
<td>CREM KO</td>
<td>EPM, OF, EZM, FC</td>
<td>−</td>
<td>+/0</td>
<td>n/a</td>
<td>↓</td>
<td>chr18 qA1 (35)</td>
<td>[270]</td>
</tr>
</tbody>
</table>

Notes: Av, Ac, Aa shown as decreased (−), increased (+), or not different (0) from wild-type (WT) controls. n/a = data not obtained by investigator. Chr = chromosome location and assigned number corresponding to illustration in Figure 1.5; OF = open-field exploration; KO = knockout; EZM = elevated-zero maze; L/D = light-dark box; EPM = elevated-plus maze; FC = fear conditioning; PA = passive avoidance; α-CaMKII = α-calcium–calmodulin kinase II; PKCγ = protein kinase Cγ; Fyn trk = Fyn tyrosine kinase; NF-kB = nuclear factor kB; GR = glucocorticoid receptor; VDR = nuclear vitamin D receptor; CREB = cAMP-responsive element binding protein; CREM = CAMP-responsive element modulator.
Neuronal messengers

Receptors and membrane-associated proteins

Intracellular signaling regulators

Figure 1.5  Chromosomal location of anxiety-related genes listed in Tables 1.2–4. (See color insert.)
analysis to mutant strains that have been generated and studied by multiple groups and to those that, although analyzed by a single laboratory, showed anxiety-related phenotypes in at least two independent behavior tests.

1.9 KNOCKOUT MICE WITH DISTURBANCES IN NEURONAL MESSENGERS EXHIBITING ALTERATIONS IN ANXIETY-LIKE BEHAVIOR

Classical neurotransmitters (GABA, NE, 5-HT) and neuropeptides have long been implicated in anxiety, so it was not surprising that inactivation of genes encoding enzymes responsible for the synthesis and metabolism of neurotransmitters or encoding neuropeptides alter anxiety levels (Table 1.2).

Glutamic acid decarboxylase (GAD) catalyzes the synthesis of GABA from glutamate and the genetic inactivation of the 65-kDa isoform of GAD (GAD65) results in anxiety-like behavior in mice [164]. Although GAD65 is responsible for the synthesis of a smaller pool of GABA than GAD67 [165, 166], it is associated with nerve terminals and synaptic vesicles and can be rapidly activated in times of high GABA demand. In GAD65<sup>−/−</sup> tissues the overall GABA content is normal but K<sup>+</sup>-stimulated GABA release is reduced. Therefore, GAD65<sup>−/−</sup> mice show no overt developmental phenotype but the more subtle anxiety-like behavioral phenotype [164] and increased seizure sensitivity [165]. In contrast, GAD67<sup>−/−</sup> mice, although born at the expected frequency, die of severe cleft palate during the first morning after birth [166]. These data are consistent with reports that enhancing synaptic GABA levels, for example by GABA reuptake inhibitors, has an anxiolytic effect [167] and indicate that an appropriate level of synaptic GABA release is important for maintaining normal behavioral responses in anxiety-inducing situations.

The enzyme catechol-O-methyl transferase (COMT) is involved in the degradation of DA, NE, and epinephrine and its inactivation also leads to anxiety-like behavior [168]. Measurement of tissue catecholamine levels in COMT<sup>−/−</sup> mice showed a specific increase in DA levels with no change in NE or 5-HT levels. Furthermore, this increase in DA seems to be restricted to the frontal cortex. Although the increased DA levels were evident in both males and females, an increased anxiety behavior was observed only in females.

Genetic inactivation of the NE transporter (NET), as expected, results in a significant increase of extracellular levels of NE [169]. These mice show increased activity in the open field that would be consistent with a reduced level of anxiety. However, no data are available regarding avoidance and autonomic arousal of these mice and hyperactivity can arouse independently of a change in anxiety.

Since 5-HTT (s/s genotype) has been identified as a susceptibility gene for anxiety (see Section 1.7), it was expected that mice with an inactivated copy of the corresponding gene would show elevated levels of anxiety. Initial studies indicated that knockout mice have no obvious behavioral phenotype even if 5-HTT binding sites were completely absent in these animals [170]. However, a later study indicated an increased anxiety-like phenotype in 5-HTT knockout mice which was more pronounced in females [171]. A more recent analysis of these mice found no differences in anxiety-related behaviors in the open-field and EPM tests, but an increase was seen in latency to feed in a novel environment [172]. Lack of a reproducible and robust anxiety-like phenotype in 5-HTT knockout mice raises the
question of how a partial reduction in 5-HTT activity in humans (s/s genotype) can be associated with elevated levels of neuroticism and anxiety.

Pharmacological experiments indicate the involvement of neuropeptides in the pathogenesis of anxiety, and this notion has been further supported by genetically modified mouse strains. An anxiety-like phenotype has been described in transgenic mice overexpressing CRH [173, 174]. However, mice with a deleted CRH gene did not differ from wild-type animals in anxiety-related behaviors even if they had significantly decreased basal corticosterone levels [175, 176]. One possible explanation is the redundancy in the central CRH system (e.g., urocortin). Consistent with this notion, stress-induced behavioral effects in CRH mutant mice could be reduced by the administration of a CRH antagonist [177]. Two independent groups have generated mice with a deletion of the urocortin gene [178], but only one study found behavioral abnormalities, namely an increased anxiety-like phenotype [178]. Finally, deletion of the CRH binding protein (BP), which normally binds and inactivates CRH, resulted in increased anxiety [179]. The authors hypothesized that the inactivation of CRH-BP may increase the “free” or unbound levels of CRH or urocortin, which results in anxiety. These data are also consistent with the reduced anxiety-like phenotype of mice constitutively overexpressing CRH-BP in the anterior pituitary gland [180].

Mice lacking the gene for NPY show a decrease in central area activity in the open field and an increased reactivity to acoustic startle [181]. However, no change in EPM was seen in NPY−/− mice compared to controls, suggesting that the absence of the peptide results in a condition characterized by increased stress responsiveness rather than anxiety.

Apart from altered pain responses, preproenkephalin-deficient mice exhibit increased anxiety in the open field and EZM [182]. It is believed that the modulatory role of enkephalins on anxiety behavior may be mediated by the GABA system [183].

Consistent with the anxiolytic activity of orphanin FQ/nociceptin (OFQ/N) or selective synthetic agonists in rodents, OFQ/N knockout mice display increased anxiety in several anxiety-related tests and impaired adaptation to repeated stress [184, 185]. Increased plasma corticosterone levels and a failure to show stress adaptation of OFQ/N knockout mice may suggest that activation of the HPA axis contributes to the anxiety phenotype in these mice.

BDNF, a member of the family of neurotrophins, promotes the formation, maturation, and stabilization of both glutamatergic and GABAergic synapses during CNS development, and it therefore regulates the balance between excitatory and inhibitory transmission, a fundamental step in neural circuit formation [186, 187]. Although homozygote BDNF knockout mice die during the second postnatal week [180, 188], heterozygote or conditional knockout mice show signs of altered emotional behavior. The role of BDNF in emotional reactions is important because the val allele has been associated with predisposition to anxiety disorders [10, 11]. Heterozygote BDNF knockout mice showed a slower escape behavior in the learned helplessness paradigm after training as compared to wild-type mice [189]. However, this effect may have been due to reduced sensitivity to centrally mediated pain as BDNF is essential for the survival and maintenance of peripheral sensory neurons [180, 188]. On the other hand, conditional BDNF mutant mice have also shown other signs of anxiety-like behavior [190]. In these conditional knockout mice, BDNF was removed after birth when most neurons are postmitotic, suggesting that the
abnormal behaviors are related to neuronal maturation, survival, and/or plasticity rather than to the absence of BDNF during behavioral testing. Recently a mouse strain was generated in which the val allele was replaced by the met allele [190a]. Met substitution for Val in BDNF is a common polymorphism in humans associated with alterations in brain anatomy and memory. In agreement with association studies [10], BDNF(Met/Met) mice exhibited increased anxiety-related behaviors indicating that this variant predisposes to anxiety disorders.

In agreement with the anxiogenic effect of intracerebroventricularly administered TNFα in the EPM [191], transgenic mice overexpressing TNFα show less exploration in novel environment [192] and increased activity [193]. However, TNFα knockout mice have a similar behavior in the EPM [193, 194]; thus, the role of TNF in the regulation of emotionality is not clear. Lack of another cytokine, IFNγ, has been reported to cause an anxiety-like phenotype [195]. However, the expression of this phenotype was visible only in C57BL/6 but not in the BALB/c mouse strain, indicating that major genetic modifiers play a role in the manifestation of anxiety in these mice. IFNγ is involved in regulating the growth of axodendritic processes, raising the possibility that, similar to the BDNF knockout mice, a neurodevelopmental abnormality underlies the anxiety in these knockout mice.

1.10 KNOCKOUT MICE WITH DEFICITS IN NEUROTRANSMITTER RECEPTORS AND OTHER CYTOPLASMIC MEMBRANE–ASSOCIATED PROTEINS EXHIBITING ANXIETY-LIKE BEHAVIOR

Blocking GABAA receptor increases anxiety-like behavior and genetic inactivation of some of the subunit genes has a similar effect (Table 1.3). For example, heterozygote $\gamma_2^{+/-}$ mice have reduced numbers of GABAA receptors and display an anxiety phenotype [107, 196]. The $\gamma_2^{+/-}$ mouse spends less time in the open arms of the EPM and less time in the lit area of the light–dark box, typical of increased anxiety-type behavior. In addition, $\gamma_2^{+/-}$ mice show increased responses in the passive avoidance paradigm. This is consistent with enhanced emotional memory for negative associations, a common feature of several anxiety disorders. These behavioral alterations are associated with a lower single-channel conductance, a pronounced deficit of functional receptors, and a reduction in $\alpha_2$-gephyrin containing postsynaptic GABAA receptor clusters in cortex, hippocampus, and thalamus. Transgenic mice overexpressing either the mouse $\gamma_2L$ or $\gamma_2S$ subunits of the GABAA receptor showed no difference in anxiety-related behavior as compared to wild-type littermates [197]. Since compensation at the level of GABAA receptor subunit expression and assembly often occurs when subunit expression is disturbed (see below), it would be important to know the expression of all subunits in these mice. Among the $\alpha$ subunits, $\alpha_1$ is predominant in GABAA receptors [198]. Two groups have independently generated mice with a deleted $\alpha_1$ subunit and found no evidence for increased anxiety or other behavioral abnormalities [199–201]. However, an additional study demonstrated that lack of the $\alpha_1$ subunit is compensated and substituted by other $\alpha$ subunits, presumably during development, mitigating the effect of the genetic deletion [199]. Although $\alpha_2$-subunit-deficient mice have been generated and a point mutation in this subunit (H101R) abolishes the anxiolytic effect of diazepam [202, 203], no published data exist on the behavior of these mice.
except a faster habituation to a novel environment of the \( \alpha_2 \)-subunit-deficient mice (which was interpreted as less activity in a novel environment) \([204]\). Therefore it is not clear if these mice have an increased anxiety phenotype. An additional complication of the interpretation of the role of the \( \alpha_2 \)-subunit can be compensation by other \( \alpha \)-subunits as occurs in the \( \alpha_{1,2} \)-subunit-deficient mice (see above). “Knockin” mice in the \( \alpha_5 \)-subunit (H105R) display enhanced trace fear conditioning to threat cues \([196]\). This is somewhat surprising because similar knockouts in the \( \alpha_{1,2} \)-subunits have no behavioral problems (see above). Further analysis showed that the knockin mice exhibit a 33% reduction in hippocampal (CA1 and CA3) \( \alpha_5 \)-receptor subunits; thus, these mice should be considered a partial knockout \([196]\). Also, \( \alpha_5 \)-receptor subunit null mutant mice exhibit improved performance in the water maze of spatial learning task but no change in locomotor activity in a novel environment \([205]\). Although the behavioral characterization of these mice is far from complete, it seems that the \( \alpha_5 \)-subunit is involved in hippocampal memory rather than in anxiety-related processes. Finally, the genetic inactivation of \( \beta_2 \), another predominant subunit, resulted in a more than 50% reduction in the total number of GAB\( \alpha \_A \) receptors and increased locomotor activity in the open field, suggesting that these receptors may control motor activity \([200]\).

Besides the GAB\( \alpha \_A \) receptor, the 5-HT\(_{1A} \) receptor has long been implicated in the pathogenesis of anxiety disorders. In 1998, three groups reported the generation of 5-HT\(_{1A} \) receptor knockout mice on different strain backgrounds \([38, 206–209]\). All three groups reported that the mutant mice exhibit consistently enhanced anxiety-like behaviors alongside reduced immobility in the forced-swim test \([209]\) or tail suspension test \([207, 208]\), indicating an antidepressant-like effect. Anxiety-related tests in these studies included open field, EPM and EZM, and novelty-induced suppression of feeding as well as fear-conditioning paradigms \([207–210]\). The consistency in these reports is rather remarkable because of the difference in the targeting constructs and genetic backgrounds. 5-HT\(_{1A} \) receptors are expressed both at postsynaptic locations in 5-HT target areas (such as amygdala, hippocampus, and cortex) and presynaptically on 5-HT neurons in the raphe nuclei as somatodendritic autoreceptors. Since autoreceptors control neuronal firing, it was first believed that the anxiety phenotype of the 5-HT\(_{1A} \) receptor knockout mice was the result of an increase in 5-HT release and activation of other 5-HT receptor subtypes. However, basal 5-HT levels are not altered, as measured by in vivo microdialysis, in 5-HT\(_{1A} \) receptor null mice \([47, 211–213]\), and expression of 5-HT\(_{1A} \) receptors in forebrain regions rescued the phenotype of 5-HT\(_{1A} \) receptor knockout mice \([214]\), suggesting that the behavioral phenotype results from the absence of postsynaptic 5-HT\(_{1A} \) receptors. Another interesting feature of the 5-HT\(_{1A} \) receptor knockout mice is that their anxiety-like behavior is likely the result of an irreversible early postnatal developmental abnormality \([214]\). In addition to increased avoidance, 5-HT\(_{1A} \) receptor knockout mice display reduced locomotor activity, another sign of increased anxiety-like behavior \([214]\). Another characteristic of anxiety, increased autonomic arousal (Figs. 1.2 and 1.3), was also observed in these mice. Specifically, following exposure to injection or novelty-induced stress, 5-HT\(_{1A} \) receptor knockout mice exhibited a significantly greater increase in heart rate and body temperature than wild-type mice \([215, 216]\). Another group reported a similar effect following footshock \([37]\). Taken together, 5-HT\(_{1A} \) receptor knockout mice show abnormalities in three important measures of anxiety: increased avoidance, decreased locomotor activity.
activity, and increased autonomic arousal following exposure to a novel environment or stress. Moreover, these behavioral changes are reproducible across laboratories, which makes this genetic anxiety model not only one of the best studied but also the most robust so far in terms of the behavioral phenotype.

Another member of the 5-HT receptor family whose deletion has been associated with an alteration in anxiety levels is the 5-HT_{1B} receptor. 5-HT_{1B} receptors are predominantly localized to nerve terminals and serve as both auto- and hetero-receptors to inhibit neurotransmitter release [217, 218]. The open-field test indicated reduced anxiety-like behavior in 5-HT_{1B} receptor knockout mice [219], suggesting that this receptor may have an opposite function than that of the 5-HT_{1A} receptor (see above). Reduced anxiety was also seen in the novelty-induced suppression of feeding test [219]. However, the light–dark box and EPM tests showed no significant change in anxiety-like behavior in the 5-HT_{1B} receptor knockout mice [94, 220]. A further complication with this strain is that its behavioral phenotype was not reproducible in different laboratories even if the source of the mice was identical [220]. A similar reduced anxiety-like behavior was recently reported in 5-HT_{2A} receptor deficient mice in the EPM, open field and the light-dark box test [220a]. Importantly, the selective cortical re-expression of the 5-HT_{2A} receptor rescued the reduced anxiety-like behavior of 5-HT_{2A} receptor knockout mice indicating a role for cortical 5-HT_{2A} receptors in the modulation of conflict based anxiety-related behavior [220a].

There are two known CRH receptors (R1 and R2) and both have been suggested to be important in regulating anxiety levels. As discussed above, there are two ligands for these receptors: CRH and urocortin. Both CRH and urocortin are potent mediators of the endocrine, autonomic, behavioral, and immune responses to stress [221, 222]. CRH-R1 has a widespread distribution with high levels in anterior pituitary, hippocampus, amygdala, and cerebellum. While in the anterior pituitary CRH-R1 is involved in the activation of the HPA axis, in other regions it is responsible for the central action of CRH/urocortin and its activation is anxiogenic. In contrast to CRH-R1, expression of CRH-R2 in the CNS is restricted to the lateral septum and the ventromedial nucleus of the hypothalamus. While mice lacking CRH-R1 display decreased anxiety in the light–dark box and the EPM [223, 224], CRH-R2-deficient mice, generated independently by three groups, exhibit varying degrees of anxiety-related behavior. Bale et al. reported an increased anxiety in the EPM and open field but not in the light–dark box test in CRH-R2-deficient mice [225]. In the study of Kishimoto et al. [226], only male CRH-R2^{-/-} mice exhibited anxious behavior in the EPM and light–dark box but, paradoxically, spent more time in the center of the open field, which is more consistent with reduced anxiety. However, Coste et al. found no significant change in anxiety behavior in the EPM or open field [227]. Although not all studies are consistent with a simple interpretation, the behavioral data obtained with various CRH-R knockout mice indicate that CRH and/or urocortin mediate a dual modulation of anxiety behavior. Activation of CRH-R1 appears to be anxiogenic while activation of CRH-R2 is anxiolytic. Therefore it may not be surprising that dual CRH-R1/2 knockout mice have only a subtle behavioral phenotype; specifically, females have a reduced anxiety-like behavior in the EPM but not in the open field while males show no behavioral abnormalities related to anxiety at all [228].
As mentioned earlier, DA, presumably by regulating reward and activity, is believed to be involved in anxiety-like behavior. In particular, DA D2 receptors have been implicated in anxiety-related behavior. Consistent with these data, DA D3 receptor knockout mice display reduced anxiety in the open field and EPM and increased locomotor activity [229]. In contrast, D4 knockout mice exhibit enhanced anxiety in the open-field test in the presence of a novel object [230]. While altered anxiety levels were evident, the authors interpreted much of the behavioral phenotype as changes in exploratory behavior.

Although a role for the cannabinoid 1 (CB-1) receptor is less known in anxiety-like behavior, it has been reported that CB-1 knockout mice have increased anxiety-like behavior in the light–dark box [231] and reduced exploration of the open arms of the EPM apparatus [232]. However, evidence for reduced anxiety was found in CB-1 knockout mice in the shock-probe burying test, in which anxiety is reflected by increased burying, corresponding to increased active avoidance [233].

Although the NE system and the locus ceruleus (LC) are clearly significant in the pathogenesis of anxiety as well as in animal models of anxiety, there are relatively few studies that specifically tested the role of adrenergic receptors in these conditions. So far, the \(\alpha_{2a}\)-adrenergic receptor has been studied (among the \(\alpha_{2a}, \alpha_{2b},\) and \(\alpha_{2c}\) receptors) and mice deficient in this receptor show increased anxiety-like phenotype in various tests [234].

Consistent with the “calming” effects of adenosine and anxiety-inducing nature of caffeine, rats treated with a nonspecific antagonist at adenosine receptors [235] as well as adenosine2a (A2a) receptor null mice exhibit increased avoidance in the EPM and light–dark box, decreased exploratory behavior, and decreased locomotor activity (reduced activity), typical signs of increased emotionality and anxiety [236] (see also Fig. 1.3). The A2a receptor is co expressed with DA D2 receptors in GABAergic neurons in basal ganglia and striatum and is thought to regulate the expression of the proenkephalin gene [237]. In situ hybridization studies showed a decrease in proenkephalin gene expression in the A2a receptor knockout mice, which may explain the anxiety-like behavior of these mice.

G-protein-gated inwardly rectifying K\(^+\) (GIRK) channels contribute to postsynaptic inhibition triggered by many neurotransmitters, including DA and 5-HT, and GIRK2-deficient mice have been found to display a phenotype consistent with reduced anxiety [238]. Four GIRK subunits (GIRK1 to GIRK4) have been identified, and tetrameric channels formed by various combinations of GIRK1, GIRK2, and GIRK3 mediate inhibition in the nervous system [239, 240]. In addition to less avoidance in the EPM and light–dark box test, GIRK2 knockout mice also display increased locomotor activity satisfying two criteria of reduced emotionality (see Fig. 1.3).

Nicotinic agonists and antagonists can modulate anxiety [241, 242], and mice with a null mutation in the nicotinic acetylcholine receptor (nAChR) \(\alpha_{7}\) subunit gene have been shown to exhibit decreased anxiety in the open field but not in the light–dark box [243]. In contrast, mice deficient in the nAChR \(\alpha_{4}\)-subunit gene display increased anxiety in the EPM [244], indicating that the subunit composition of the nAChR may determine whether the effect is anxiogenic or anxiolytic.

One of the targets of BDNF is trkB, a receptor tyrosine kinase [245]. Consistent with the increased anxiety-like phenotype of the conditional BDNF mutant mice
transgenic mice overexpressing trkB in postmitotic neurons in a pattern similar to that of the endogenous receptor display less anxiety in the EPM test [246]. Neurotransmitter and neuromodulator receptors are not the only substrates of communicating external signals into neurons. Cell–cell interactions are crucial in regulating neuronal functions and developmental processes. One group of proteins that mediate cell–cell interactions is represented by neuronal adhesion molecules that regulate, among others, synaptic plasticity in both the developing and adult brain. Recent studies indicate that neuronal cell adhesion molecules of the immunoglobulin superfamily (NCAM and L1) are important mediators of the effects of stress. Chronic stress alters the expression pattern of cell adhesion molecules in parallel with their effects on behavior [247]. The connection between neuronal cell adhesion molecules and emotional behavior is also supported by the change in the anxiety-like phenotype of NCAM and L1 null mice. Genetic inactivation of NCAM results in decreased anxiety in the light–dark and EPM tests [248]. In addition, these mice respond to the anxiolytic effect of buspirone in the light–dark test at lower doses than the NCAM$^{+/+}$ mice, suggesting that there may be an alteration in the sensitivity of the 5-HT$_{1A}$ receptors in these knockout mice. However, the authors reported no changes in the density of 5-HT$_{1A}$ receptors or in tissue 5-HT content. Since NCAM has been demonstrated to have a role in CNS development and neuroplasticity, a developmental abnormality may explain the expression of anxiety-like behavior in these mice (similarly to the BDNF, IFN$\gamma$, and 5-HT$_{1A}$ knockout mice; see Sections 1.9 and 1.10). Also, conditional inactivation of L1 in the forebrain, mostly from early postnatal life [by cre-recombinase under the control of the calcium/calmodulin-dependent protein kinase II (CaMK II) promoter], resulted in decreased anxiety in the open field and EPM [249]. Conditional expression avoids the severe morphological and behavioral abnormalities associated with the absence of L1 during prenatal development. Finally, the lack of cadherin-11, another cell adhesion molecule, results in reduced fear- or anxiety-related responses [250]. Cadherin-11 is expressed in the limbic system of the brain, most strongly in the hippocampus, and is densely distributed in synaptic neuropil zones. Taken together, the loss of function of three cell adhesion molecules leads to maladaptive behavioral responses that are “opposite” to anxiety and may be characterized as excessive novelty seeking and a lack of appropriate response to danger (see Fig. 1.3, blue region of the cube, and discussion in the accompanying text). It is striking that all three adhesion molecules mentioned above are involved in the regulation of synaptic structure and function [251]. This indicates that the optimal functioning of synapses is essential for mediating appropriate responses to novelty and stress.

1.11 INTRACELLULAR REGULATORS ASSOCIATED WITH ANXIETY-LIKE PHENOTYPE

A number of intracellular signaling molecules and transcription factors have been shown to cause increased or reduced anxiety-like phenotype in mice. The $\alpha$ isoform of CaMKII is an important second messenger, and Chen et al. [252] demonstrated decreased anxiety in CaMKII knockout mice. CaMKII is a major component of the postsynaptic density in glutamatergic synapses [253] and is involved in neuronal
functions related to calcium signaling, including the induction of long-term potentiation (LTP) [254]. Therefore, the disruption of CaMKII function could alter many aspects of neuronal function, making it difficult to relate it to a specific anxiety behavior. Indeed these knockout mice also exhibit enhanced aggression and learning impairment.

The serine/threonine kinase protein kinase C γ (PKCγ) has recently been shown to be a regulator of anxiety behaviors [255]. PKCγ is restricted to the CNS and is highly expressed in limbic areas of the brain. In three different behavioral tests (EPM, light–dark test, mirrored chamber) PKCγ knockout mice consistently showed reduced anxiety-like behavior. Bowers et al. [255] proposed that PKCγ modulates anxiety by altering the function of GABA_A, N-methyl-D-aspartate (NMDA), or 5-HT_2 receptors.

Another intracellular signaling molecule implicated in anxiety and fear responses is the tyrosine kinase Fyn. Fyn is a member of the Src family of tyrosine kinases that can associate with and phosphorylate a variety of molecules. Inactivation of the fyn gene in mice results in increased anxiety-like behavior to naturally aversive stimuli in the light–dark box and novelty tests [256]. These mice also display enhanced learned fear responses in the passive-avoidance test. Fyn is highly expressed in the limbic system and has been implicated in NMDA receptor–mediated synaptic plasticity, NCAM-dependent neurite outgrowth, and myelination [257–259]. Whether any or all of these processes are involved in the enhanced anxiety exhibited in the Fyn^-/- mice is unclear.

The NF-kB transcription factor family is linked to a number of receptors, including TNFα, and controls the expression of many genes involved in cell survival, proliferation, and regulation of inflammatory and stress responses. It has recently been shown that mice lacking the p50 subunit of NF-kB have a reduced anxiety-like phenotype [260]. These mutant mice showed reduced avoidance and autonomic arousal in the open field and EPM. In immune cells, NF-kB factors are kept inactive by association with inhibitory proteins belonging to the IkB family and activating stimuli induce the phosphorylation, polyubiquitination, and proteasome degradation of IkBs, allowing NF-kB to translocate into the nucleus and activate target genes. In contrast, it seems that either NF-kB is constitutively active in neurons [261] or normal neuronal activity is sufficient to keep a substantial amount of NF-kB in an active form. Since it is expressed during development [262], NF-kB may regulate the development of brain circuits, and consequently the reduced anxiety-like phenotype of p50 knockout mice could be due to abnormal brain development.

GR is another transcription factor (activated via the HPA axis and glucocorticoid hormones) and is well known to be involved in stress response and some anxiety disorders. As discussed later, a brief period of controllable stress experienced with general arousal and excitement can be beneficial, but chronically elevated levels of circulating corticosteroids are believed to enhance vulnerability to a variety of diseases, including affective disorders. Therefore it is not surprising that the genetic manipulation of GR results in changes in emotionality in mice. Reduced anxiety-like phenotype was found in a brain-specific GR knockout [263] and in a GR-antisense model with reduced GR expression in brain and some peripheral tissues [264], while GR overexpression in forebrain results in increased anxiety-like behavior [265]. Together, these findings indicate that a sustained increase in GR activity in brain is associated with increased anxiety-like behavior.
In addition to its role in the regulation of calcium and phosphate homeostasis and in bone formation, vitamin D is also thought to be involved in brain function. Genetic ablation of the vitamin D receptor (VDR), another nuclear receptor linked to transcription, results in increased anxiety-like behavior in a battery of behavioral tests [266].

Still another group of transcription factors associated with anxiety-like behavior is the family of cyclic adenosine monophosphate (cAMP)-responsive nuclear factors that consist of CREB, CRE modulator (CREM), and activating transcription factor 1 (ATF-1) [267]. A conditional CREB mutation that inactivates all isoforms in the brain or the disruption of the two major transcriptionally active CREB isoforms (α and δ) increases anxiety-like responses in mice in different behavioral tests, including the EPM [268]. In CREB-deficient mice, the expression of CREM isoforms is increased [269]; thus, the higher anxiety-like phenotype may be attributable to this change. Indeed, CREM-deficient mice display reduced anxiety-like behavior in the EPM test and also exhibit hyperactivity [270], indicating that CREM activity may be linked to neuronal modulation promoting anxiety.

BC1 RNA is a small non–messenger RNA common in dendritic microdomains of neurons in rodents, and it is believed to play a role in translational regulation. Mice mutant for BC1 show behavioral changes consistent with increased anxiety and reduced exploration [271]. These data indicate that an anxiety-like phenotype can be induced by disturbing gene expression beyond transcription at the translational level.

Taken together, defects in intracellular processes involving second messengers, transcription factors, and translational factors can lead to alterations in anxiety in mice. To better understand the neurobiology of anxiety, it will be critical to identify the specific mRNAs and proteins whose altered synthesis in neurons of the fear/anxiety pathway is associated with the expression of the behavioral phenotype. In any event, the common feature of these molecular changes is that they could all eventually influence morphological and/or functional plasticity in the nervous system (see further discussion on this topic below).

1.11.1 Modeling Complex Genetics of Anxiety in Mice: Oligogenic Anxiety-Like Conditions in Mice

Genetic studies on various mouse phenotypes clearly indicate that most behavioral traits are heritable and are specified by multiple genes or QTLs. For example, mapping studies have estimated that the individual anxiety-related behavioral differences in the DeFries recombinant inbred strains of mice are the result of the interaction between four to six QTLs for each behavior; the largest QTL explains no more than half of the variance attributable to the detected QTL [15, 272, 273]. However, QTLs can consist of hundreds of genes, and these studies are not designed to analyze the contribution of individual genes to anxiety. An alternative strategy to study the combined effect of two or more genes on behavior is to use double- and triple-knockout mice. A recent report analyzed anxiety-related behaviors in double 5-HTT−/− and BDNF+/− mutant mice [274]. These mice, as compared to 5-HTT−/−, BDNF+/−, and wild-type mice, displayed a significantly higher level of anxiety-like behavior, reduced levels of 5-HT and 5-hydroxyindole acetic acid in the hippocampus and hypothalamus, and greater increases in plasma ACTH after a
stressful stimulus. These findings support the hypothesis that genetic changes in BDNF expression interact with 5-HT to modulate anxiety and stress-related behaviors.

Another double-knockout strain lacking both monoamine oxidase (MAO) A and B, two enzymes responsible for the degradation of monoamines, shows anxiety-like behavior in various tests [275]. Neither the MAOA nor the MAOB knockout mutants display anxiety in these tests, indicating that an interaction between the two MAO genes leads to a novel phenotype [276, 277]. Since monoamine levels are higher in the double knockouts than in the single mutants, the abnormal behavior of MAOA/B mutants is likely the consequence of altered monoaminergic neurotransmission.

1.12 EFFECTS OF EARLY-LIFE ENVIRONMENT ON ANXIETY

1.12.1 Early-Life Experience on Expression of Anxiety in Later Life

A large body of evidence supports the notion that early-life environmental effects alter life-long stress-coping mechanisms. Unlike human studies, which are predominantly retrospective with a large number of environmental variables, animal research has focused on the effect of “handling” and maternal care during postnatal development. Brief handling of pups results in life-long decreases in behavioral and endocrine responses to stress while animals separated from their mothers/litters for longer periods of time (i.e., for several hours) exhibit increased anxiety [278–280]. Later studies determined that the critical feature of short-term handling was the increase in maternal care [licking and grooming (LG)] following the return of pups [281]. Variability in maternal care can produce large differences in adult behavior and hormonal responsiveness to stress. Pups nursed by mothers with either a high or a low level of LG and arched-back nursing (ABN) show a decreased and increased level of anxiety-like behavior in adult life (open field, novelty-suppressed feeding, and shock-probe burying assays), respectively [281–284]. Also, offspring of high LG–ABN mothers (as well as briefly handled pups) have reduced plasma levels of ACTH and corticosterone in adulthood following stressful stimuli such as restraint stress when compared to the offspring of low LG–ABN mothers (or nonhandled pups) [285]. Furthermore, these animals show increased glucocorticoid feedback sensitivity, increased hippocampal GR mRNA expression, and decreased hypothalamic CRH mRNA levels.

Interestingly, pups born to low LG–ABN mothers but cross fostered to high LG–ABN mothers develop low anxiety-like behaviors in adulthood, but high LG–ABN pups reared by low LG–ABN mothers do not develop increased anxiety-like responses in adulthood [285]. Furthermore, the maternal behavior of female offspring from low LG–ABN mothers can be changed by cross fostering them to high LG–ABN mothers. The reverse, however, is not true because daughters of high LG–ABN mothers raised by low LG–ABN dams have high LG–ABN maternal behavior [281, 286]. Finally, offspring of low LG–ABN mothers, if cross fostered to high LG–ABN mothers, show hormonal levels similar to those observed for offspring of high LG–ABN mothers [285]. These findings suggest that environmental effects may overpower genetic predispositions, particularly in cases where such modification would be beneficial for survival.

Although experiments related to both postnatal handling and maternal behavior clearly show a nongenomic influence on anxiety-like behavior, the transmission
mechanism of this effect has been difficult to elucidate. It has been hypothesized that environmental influences exert some level of control on the development of HPA and the regulation of HPA function via a number of neurotransmitter systems, including the noradrenergic, GABAergic, and glutamatergic systems [287, 288]. For example, rat pups of high LG–ABN dams show altered GABA\(_A\) receptor subunit expression in the amygdala, LC, medial prefrontal cortex, and hippocampus that could contribute to their reduced anxiety-like behavior as compared to pups from low LG–ABN dams [289, 290]. In addition to the GABAergic system, other potential factors mediating the environmental effects include the glutamatergic system and neurotrophins such as BDNF. Liu et al. [291] found that adult offspring of high LG–ABN Long Evans dams show increased hippocampal synaptogenesis and better spatial learning and memory than low LG–ABN offspring and that these differences could be equalized if low LG–ABN pups were cross fostered to high LG–ABN dams. More specifically, increased LG–ABN of offspring resulted in increased hippocampal mRNA expression of NR2A and NR2B NMDA receptor subunits at postnatal day 8, a change that was sustained into adulthood. Consistent with the regulation of the BDNF gene by NMDA receptors [292], increased levels of BDNF, but not NGF or NT-3, mRNA were observed in the dorsal hippocampus of eight-day-old high LG–ABN pups [291]. Most recently, it has been reported that the maternal effect is linked to alterations in methylation and chromatin structure at the GR promoter in the offspring [291a]. It has been proposed that downregulation of hippocampal GR in the pups of low nursing mothers compromises feedback inhibition in the hypothalamic pituitary adrenal axis ultimately leading to higher anxiety states [291a]. Since GR knockout mice have reduced anxiety [263] and GR overexpression in forebrain results in increased anxiety-like behavior [265], it is possible that the maternally-induced regulation is specific for a subset of GRs and does not involve the GR pool implicated in the stress related actions of glucocorticoids.

1.12.2 Interaction of Environment with Genes in Establishing Level of Anxiety

Although the interaction of genes and environment in shaping personality is well accepted, direct experimental evidence to support this notion has been difficult to obtain in humans. Recent association studies, however, have clearly indicated that genetic and environmental factors act together, enhancing the phenotype beyond the level established by either factor alone. For example, promoter polymorphism in 5-HTT can influence anxiety-related behavior (s/s genotype represents a predisposition to neuroticism/anxiety) [9], and a recent report showed that this polymorphism moderates the influence of stressful life events on depression. Individuals with one or two copies of the s allele of the 5-HTT promoter polymorphism exhibited more depressive symptoms in relation to stressful life events than l/s individuals [293]. Although this study was focused on depression, anxiety is a common symptom in depression, and future studies may reveal evidence of an interaction between an individual’s 5-HTT allelic makeup and environmental insults in anxiety disorders. This connection has already been made in primates. Rhesus monkeys have a 5-HTT polymorphism similar to that found in humans, and it was shown that although both mother-reared and nursery-reared heterozygote (l/s) animals demonstrate increased affective responding (a measure of temperament) relative to l/l homozygotes,
nursery-reared but not mother-reared l/s infants exhibited lower orientation scores than their l/l counterparts [294]. Also, monkeys with deleterious early rearing experiences were differentiated by genotype in cerebrospinal fluid concentrations of the 5-HT metabolite, 5-hydroxyindoleacetic acid, while monkeys reared normally were not [295]. Another study found that separation-induced increases in ACTH levels were modulated by both rearing condition and 5-HTT polymorphism [296]. During separation, animals with l/s genotypes had higher ACTH levels than l/l animals, and peer-reared l/s animals had higher ACTH levels than all other groups, including mother-reared animals.

Rodents are more amenable to such studies, and there have been a number of reports on the effect of early-life experience and gene interaction on later-life behavior. For example, early-life handling or cross fostering of highly neophobic BALB/c mice to less neophobic C57BL/6 mice equalizes both the behavioral and the benzodiazepine receptor expression differences between these two strains as well as decreasing the ACTH release following an acute stressor of BALB/c mice in adulthood [284, 297–299]. Furthermore, the effect of the 5HT1A receptor gene on the anxiety-like behavior may be modulated by the environment. In a recent study, Weller et al. documented that F1 5HT1AR+/− offspring reared by 5HT1AR−/− mothers have increased ultrasonic vocalization (USV) when compared to F1 5HT1AR+/− offspring reared by 5HT1AR+/+ dams [300]. However, contrary to expectations, F1 5HT1AR+/- offspring reared by 5HT1AR−/− mothers have decreased measures of anxiety in the EPM as adults when compared to F1 5HT1AR+/- offspring raised by 5HT1AR+/+ dams. Also, 5HT1AR−/− pups reared by either 5HT1AR−/− or 5HT1AR+/- dams produced less isolation-induced response (USV) than their 5HT1AR+/+ controls [300]. Although it is difficult to consolidate these seemingly contradictory results, these experiments show that the level of anxiety associated with 5HT1AR deficiency can be altered by environmental factors.

In addition to early environmental influences, later-life or adult environment can also influence the expression of emotionality, as demonstrated in mouse models. As discussed previously, lack of the nociceptin/orphanin FQ gene leads to an enhanced anxiety phenotype in mice [184, 185]. The strength of the behavioral expression of the phenotype is dependent, however, on environmental influences such as social interactions. Ouagazzal et al. found that homozygous mutant animals, when housed alone, performed similarly to their wild-type controls on tests of emotional reactivity. Enhanced emotionality became apparent only when the singly housed animals were introduced to group housing (five animals per cage) that induced greater levels of aggression and increased anxiety responses [301].

Taken together, these data show that both genetic and environmental factors have an important role in establishing emotionality in mammals. Often, these factors work together, enhancing the phenotype beyond the level established by either factor alone. Other times, the environmental influences can partially or fully rescue undesirable phenotypes caused by genetic predispositions or mutations, enhancing the likelihood of the organism’s survival.

1.13 CONCLUSIONS: NEUROBIOLOGY OF ANXIETY DISORDERS

Combining what is known about anxiety disorders (including symptomatology, pharmacology, and biochemistry) with the genetic and molecular information
gathered from the diverse knockout mouse strains that exhibit alterations in anxiety-like behavior, it becomes apparent that anxiety-related pathways and processes involve communication between neurons (including neuronal messengers and their receptors) and/or signaling within cells (Fig. 1.6 [302]). Since the manipulation of a ligand, its receptor, and a coupled intracellular signaling elicits a similar anxiety-like behavior, it is possible to cluster these molecules to pathways. Many of these pathways eventually converge onto regulation of transcription and/or translation, and one can hypothesize that anxiety-like behavior is the result of changes, at least in part, at the level of gene expression (Fig. 1.6). Indeed, the genetic manipulation of transcription (by CREB, GR, VDR, and NF-kB; see previous sections) can also result in changes in anxiety levels.

Several of these “anxiety-related pathways” can be established. For example, the “serotonergic” pathway consists of receptors controlling the release of 5-HT (5-HT1A and 5-HT1B receptors), 5-HTT, postsynaptic 5-HT receptors, and mitogen-activated protein kinase/extracellular regulated protein kinase (MAPK/ERK) signaling (Fig. 1.6). As described in this chapter, a change in any of these components can lead to altered emotional behavior. The proper function of this pathway is especially crucial during early postnatal development, and one can hypothesize that abnormal signaling via this system alters the development of neuronal networks and consequently function, manifested as abnormal fear/anxiety response. BDNF, whose deficiency has also been associated with anxiety-like behavior, is also linked to the MAPK–ERK signaling (Fig. 1.6), suggesting that the two distinct anxiety-associated traits (deficit in 5-HT1A and BDNF) may share downstream targets. In addition to the convergence of various pathways, the same extracellular signal can diverge to various intracellular pathways illustrated by the coupling of the 5-HT1A receptor to both the MAPK–ERK and NF-kB pathways [303–307] (Fig. 1.6). Such divergence obviously broadens the clusters of affected genes. Since manipulation of the p50 subunit of NF-kB (activated by cytokines) can also be linked to anxiety-like behavior, crosstalk is extensive at the signaling level and therefore within and between anxiety-related pathways. Although the function of 5-HT and BDNF pathways is altered not only as a result of mutations and genetic polymorphisms but also by the environment, another “anxiety-related” pathway consisting of CRH, ACTH, and GR is especially sensitive to environmental changes. As with the other pathways, activation of this pathway by chronic stress eventually alters gene regulation (via GR).

It is hypothesized that abnormalities in these pathways at any level lead to, via altered gene expression, changes in neuronal morphology and/or function. Indeed, a number of knockout mouse strains with an anxiety-like phenotype as well as rodents following chronic stress show altered dendritic arborization in hippocampus and amygdala [308, 309], abnormal synapse formation [126, 250, 310], and altered electrical properties of neurons [311]. These changes result in abnormal neuronal network activity characterized by a deficit in short-term plasticity (i.e., hippocampal paired pulse facilitation and inhibition) [210, 312, 313], abnormalities in long-term potentiation [250, 312], an increase in network excitability [210, 311, 314], and abnormal activation or inhibition of brain regions as measured by fMRI [74, 75, 78]. In anxiety disorders, multiple molecular pathways may be simultaneously affected in multiple brain regions, consistent with the multitude of associated symptoms. Importantly, all commonly used anxiolytic drugs [benzodiazepines, selective
Figure 1.6  Unifying model of pathogenesis of anxiety. Pharmacological and genetic studies indicate that defects in specific substrates of neuronal communication (e.g., 5-HT, GABA, 5-HT receptors), intracellular signaling (e.g., ERK), and gene expression (GR) may be involved in anxiety-related behavior. These extracellular neuronal messengers and their associated receptors and coupled intracellular signaling form specific pathways. Abnormalities in these molecular pathways at various levels can directly modify the function/morphology of the neuronal network associated with fear, ultimately producing changes in anxiety levels. (See color insert.)

serotonin reuptake inhibitors (SSRIs), and buspirone] can be integrated into the model described above, indicating that the genetic data are consistent with the pharmacological data and that anxiolytic drugs target and modulate the molecular and cellular pathways which apparently control or establish (during development) the level of anxiety (Fig. 1.6).

REFERENCES


REFERENCES


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