Chapter 21

Anxiety disorders

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Introduction

There is a bewildering diversity of tests that claim face, construct, and/or predictive validity as animal models of anxiety disorders (for a recent review see Cryan and Sweeney, 2011). Most of these procedures use rats or mice as subjects and involve exposure of subjects to external (e.g., cues earlier paired with footshock, bright light, predator) or internal (e.g., drug states) stimuli that are assumed to be capable of inducing anxiety in animals. The last two decades have seen the emergence of endophenotype models of anxiety, which are often referred to as trait anxiety tests. Endophenotypes do not vary from moment to moment and are considered to be enduring features of an individual. They are cognitive, psychological, anatomical, or biochemical traits, which are hereditary and represent reliable markers of both the disease state and disease risk (Hasler et al., 2004). It has been suggested that the endophenotypes observed in anxiety disorders represent facets of disease more amenable to the development of animal models (Gottesman and Gould, 2003). Indeed, the traits encountered in anxiety disorders such as autonomic hyperarousal, trauma-induced cognitive deficits, compulsatory behaviors, startle response, sleep disturbances, and avoidance or difficulty to escape areas can all be readily modeled (Cryan and Holmes, 2005). These models either use rodents that have been selected for emotional reactivity or employ constitutive genetic manipulations such as targeted deletion, insertion, or mutation of a gene for the purpose of altering the protein product encoded by the gene (Jacobson and Cryan, 2010).

The aim of the present chapter is to provide an overview of mouse models of anxiety disorders generated by constitutive genetic manipulations. Strains and selected lines that display endophenotypes for anxiety disorders are reviewed by Holmes in Volume 1 of this book series (Holmes, 2014). A review of the literature indicates that nearly 50 strains of mice have been generated by using gene-targeting technology, which display a phenotype consistent with increased anxiety (Table 21.1). While many of these phenotypes appear to reflect the known function of the target in emotional processes, a few others include genes that have not been shown to be involved in anxiety behaviors earlier (e.g., fyn protooncogene, mas oncogene, tumor necrosis factor-α; for an earlier review of these models see Belzung and Griebel, 2001). The focus of the current chapter will be on models involving genetic modifications of the serotonin (5-HT), GABA, and the corticotropin-releasing factor (CRF) systems, as they have been shown to play a crucial role in the modulation of anxiety behaviors.

There are several other mutants generated from the hypotheses based on the mechanisms of action of clinically efficacious anxiolytics, or to reproduce a human genetic mutation thought to be linked to anxiety. Examples include null mutations in the monoamine system such as monoamine oxidase A (MAO-A), catechol-O-methyltransferase (COMT), and norepinephrin transporter (NET). The involvement of the endocannabinoid system in anxiety behavior has likewise stimulated the generation of mutants implicated in this pathway, such as null mutants of CB1 receptors and fatty acid amide hydrolase (FAAH), the principal enzyme responsible for the degradation of the endogenous cannabinoid, anandamide. The anxiety-related phenotype of these mutants are summarized in Table 21.1, and has been reviewed elsewhere (Belzung et al., 2008; Belzung and Griebel, 2001; Cryan and Sweeney, 2011; Holmes, 2001; Jacobson and Cryan, 2010) and readers are referred to these publications for further details.

Genetic models of anxiety based on manipulations of the 5-HT system

Serotonin has long been shown to participate in the etiology and treatment of anxiety (Griebel, 1995). The most common treatments for many anxiety disorders are the selective 5-HT reuptake inhibitors (SSRIs), which are thought to exert their therapeutic effects by increasing extracellular 5-HT levels (Gartside et al., 1995), and to a lesser extent, the 5-HT_{1A} receptor partial agonists, which have been suggested to produce their anxiolytic activity by activating 5-HT_{1A} heteroreceptors in forebrain areas such as the cortex and striatum (Akimova et al., 2009; Goodfellow et al., 2009; Zhang et al., 2010). A major finding has been the discovery of genetic variation in the 5-HT transporter (5-HTT) and the 5-HT_{1A} receptor, and its influence on emotional traits. Lesch and colleagues (1996) were the first to report that

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Table 21.1 Anxiety-related phenotypes of genetic manipulation in mice.

Gene	Mut	Genetic background	Models	References
3 x Tg-AD	Tg	C57BL/6	CFS, LD, OF	Espana et al., 2010
5-HT_{1A}	KO	129/Sv, C57BL/6J	EZM, OF	Heisler et al., 1998
		Swiss-Webster/12^{SV}	OF	Parks et al., 1998
		129Sv, C57BL6/J	EPM, OF	Ramboz et al., 1998
		129Sv, Swiss-Webster	EPM, OF	Sibille et al., 2000
		129Sv, C57BL/6J	EPM, OF, NSF	Gross et al., 2002
		129Sv	SIH	Pattij et al., 2002a
		B6	EPM, OF	Bailey and Toth, 2004
		C57BL/6J	EPM	Li et al., 2004
		129Sv, C57BL/6J	CFS, LD	Klemenhagen et al., 2005
		C57BL6/J, CBA/J	CFS	Tsetsenis et al., 2007
		Swiss-Webster, B6, 129SvEv	EPM, OF	Gleason et al., 2010
		126S6/Sv, C57B6, CBA	OF, LD	Richardson-Jones et al., 2011
5-$HT_{1A/1B}$	KO	129Sv, C57BL/6	EPM, NSF, OF	Guilloux et al., 2011
5-HT_{2C}	KO	C57BL/6J, BALB/c	EPM	Mombereau et al., 2010
5-HT_3	KO	129, C57BL/6J	DW	Bhatnagar et al., 2004a
		129, C57BL/6J	CFS	Bhatnagar et al., 2004b
5-HTT	KO	C57BL/6J	EPM, ET, LD, OF	Holmes et al., 2003d
		129S6/SvEv	NSF	Lira et al., 2003
		129S6/SvEv	EPM, NSF, OF, SEP	Ansorge et al., 2004
		129P1/ReJ, C57BL6/J	EPM, LD, OF	Carroll et al., 2007
		129P1/ReJ, C57BL6/J	CFS	Wellman et al., 2007
		129P1/ReJ, C57BL/6J	EPM, LD, OF	Heiming et al., 2009
		129P1/ReJ, C57BL/6J	EPM, LD, NSF, SA	Line et al., 2011
		C57BL/6J	CFS	Narayanan et al., 2011
α-CaMKII	Tg	C57BL/6N	EZM, LD, OF, SI	Hasegawa et al., 2009
Adenosine A_{2a}	KO	CD1	EPM, LD	Ledent et al., 1997
Adrenergic α_{2a}	KO	C57BL/6	LD, OF	Schramm et al., 2001
	KO	C57BL/6J	EPM, OF, MB	Lähdesmaki et al., 2002
Angiotensin II type-2	KO	129Sv, C57BL/6	EPM, LD	Ichiki et al., 1995
	KO	129Sv, C57BL/6	LD	Okuyama et al., 1999
Apolipoprotein E	KO	C57BL/6J	EPM	Raber, 2007
APP	Tg	C57BL/6	CFS, LD, OF	Espana et al., 2010
Cannabinoid CB_1	KO	CD1	LD	Martin et al., 2002
		CD1	LD, OF	Maccarrone et al., 2002
		CD1	EPM	Haller et al., 2002
		C57BL/6JOlaHsd	CFS	Marsicano et al., 2002
		CD1	EPM, SI	Haller et al., 2004a
		C57BL/6NCrl	CFS	Kamprath et al., 2006
		CD1	EPM	Tourino et al., 2008
		C57BL6J	LD	Bura et al., 2010
		C57BL/6NCrl	CFS	Kamprath et al., 2009
		CB1, C57BL/6N	CFS, EPM	Dubreucq et al., 2012
		CB1, C57BL/6N	CFS	Metna-Laurent et al., 2012

(cont.)

Table 21.1 *(cont.)*

Gene	Mut	Genetic background	Models	References
Cannabinoid FAAH	KO	C57BL/6J	EPM	Haller et al., 2004b
COMT	KO	129, C57BL/6	LD	Gogos et al., 1998
CCK CCK-R2	KO	129Sv, C57BL/6J	EPM	Vasar et al., 2000
	KO	C57BL/6J	EPM	Miyasaka et al., 2002
	KO	129Sv, C57BL/6J	EPM	Abramov et al., 2008
	Tg	B6, CBA	CFS, OF, SI	Chen et al., 2006
CRF	Tg	C57BL/6, SJL	EPM, OF	Stenzel-Poore et al., 1994
	Tg	C57BL/6, SJL	EPM, OF	Stenzel-Poore et al., 1996
	Tg	B6, SJL	LD	Heinrichs et al., 1997b
	Tg	C57BL/6, SJL	CFS, LD	van Gaalen et al., 2002
	Tg	C57BL/6	OF, LD	Kolber et al., 2010
CRF-binding protein	KO	C57BL/6	EPM, OF	Ramesh et al., 1998
	KO	C57BL/6J	DW, EPM	Karolyi et al., 1999
CRF-R1	KO	129S2, C57BL/6J	LD	Refojo et al., 2011
CRF-R2	KO	129, C57BL/6	EPM, OF	Bale et al., 2000
	KO	129, C57BL/6	OF	Coste et al., 2000
	KO	129, C57BL/6	EPM, LD, OF	Kishimoto et al., 2000
Desert hedgehog	KO	C57BL/6, CD1	VOG	Umehara et al., 2006
Dopamine D4	KO	129, C57BL/6	OF	Dulawa et al., 1999
Estrogen α	KO	129, C57BL/6	LD	Ogawa et al., 1997
FMR1	KO	C57BL/6J	MC, SI	Spencer et al., 2005
Fyn tyrosine kinase	KO	129, C57BL/6	EPM, LD, OF	Miyakawa et al., 1994
$GABA_{A\,\alpha1}$	KO	129S1, FVB/N	CFS	Sonner et al., 2005
$GABA_{A\,\alpha2}$	KO	129SvEv, C57BL/6	CER	Dixon et al., 2008
$GABA_{A\,\beta3}$	KO	129SvJ, C57BL/6	EPM	Liljelund et al., 2005
	KO	129SvJ, C57BL/6J	EPM, MD	Hashemi et al., 2007
$GABA_A\,\gamma_2$	KO	129, C57BL/6	CFS, EPM, LD	Crestani et al., 1999
	KO	129, C57BL/6	EPM	Homanics et al., 1999
	KD	129SvJ, C57BL/6	EPM, FNE	Chandra et al., 2005
	KO	129SvJ	EPM, FET, NSF	Earnheart et al., 2007
$GABA_{B(1)}$	KO	BALB/c	EZM, ST	Mombereau et al., 2004a
	KO	BALB/c	LD	Mombereau et al., 2004b
	KO	BALB/c	LD	Mombereau et al., 2005
$GABA_{B(2)}$	KO	BALB/c	LD	Mombereau et al., 2005
GABA GAD65	KO	C57BL/6	EPM	Kash et al., 1999
	KO	C57BL/6, CBA2	LD, OF	Stork et al., 2000
	KO	C57BL/6, CBA2	CFS	Stork et al., 2003
	KO	C57BL/6	CFS	Bergado-Acosta et al., 2008
	KO	C57BL/6	CFS	Sangha et al., 2009
GABA GAT1	KO	129SvEV Tac FBR	EPM	Chiu et al., 2005
Galanin GAL-R1	KO	129Sv, C57BL/6	EPM	Holmes et al., 2003b
Glucocorticoid	Tg	C57BL/6J	EPM, LD	Wei et al., 2004
Glutamate DAO	KO	C57BL/6J	EPM, NO, OF	Labrie and Roder, 2009
Glutamate GluN2B	KI	C57BL/6J	EPM	Delawary et al., 2010

Table 21.1 *(cont.)*

Gene	Mut	Genetic background	Models	References
Glutamate mGluR4	KO	C57BL/6	EZM, OF	Davis et al., 2012
Glutamate mGluR5	KO	129Sv, C57BL/6	EPM	Wu et al., 2007
Glutamate mGluR8	KO	ICR	EPM	Linden et al., 2002
	KO	C57BL/6J	EPM, OF	Duvoisin et al., 2005
		129SvEv	EPM	Sparta et al., 2007
		129/OlaHsd, C57BL/6	EPM, OF	Robbins et al., 2007
		C57BL/6J	AS, EZM	Duvoisin et al., 2011
Hdc	KO	C57Bl6/J	EPM, LD, OF	Acevedo et al., 2006
Interferon γ	KO	129, C57BL/6	EPM	Kustova et al., 1998
Interleukin 6	KO	129, C57BL/6	EPM	Armario et al., 1998
Mas oncogene	KO	129, C57BL/6	EPM	Walther et al., 1998
Midkine	KO	129	EPM	Nakamura et al., 1998
NCAM	KO	129/Ola/Hsd, C57BL/6J	EPM, LD	Stork et al., 1999
Nicotinic α4	KO	BALB/c, C57BL/6	EPM	Ross et al., 2000
Nociceptin	Tg	129/Ola, C57BL/6	AS, LD	Ouagazzal et al., 2003
NOS	KO	C57BL/6	EPM, OF	Frisch et al., 2000
Nociceptin	KO	129, C57BL/6	EPM, LD, OF	Köster et al., 1999
Nociceptin R	KO	129, C57BL/6J, CD1	EPM, ETM, LD	Gavioli et al., 2007
NPY	KO	129, C57BL/6	EPM	Palmiter et al., 1998
	KO	129Sv, C57BL6	AS, EPM, OF	Bannon et al., 2000
	KO	129SvJ, C57BL/6	EPM, OF	Painsipp et al., 2011
	Tg	C57BL/6, DBA/2	EPM	Inui et al., 1998
NPY Y_1	KO	129SvJ, C57BL/6	LD	Karl et al., 2006
Preproenkephalin	KO	129, CD1	EPM	Konig et al., 1996
Puromycin-sensitive aminopeptidase	KO	BALB/c	EPM	Osada et al., 1999
SF1	KO	C57BL/6	EPM, LD, MB, OF	Zhao et al., 2008
Single-minded 2	Tg	129Sv, C57BL/6, SJL	EPM	Chrast et al., 2000
TRH-R2	KO	129/SvJ	NSF	Sun et al., 2009
TgActβE	Tg	C57BL/6	EPM, OF	Sekiyama et al., 2009
TgNTRK3	Tg	B6, SJL-F1J	EPM, EZM, MDTB	Dierssen et al., 2006
Tumor necrosis factor-α	Tg	C57BL/6, CBA	LD	Fiore et al., 1998
TSC-DN	Tg	?	EPM, OF	Ehninger and Silva, 2011
Vasopressin V1a	Tg	129Sv, C57BL6	LD	Bielsky et al., 2005

AS, acoustic startle; CFS, conditioned fear stress; CER, conditioned emotional response; COMT, catechol-O-methyltransferase; DAO, D-amino-acid oxidase; EPM, elevated plus maze; ET, emergence test; ETM, elevated T maze; EZM, elevated zero maze; FAAH, fatty acid amide hydrolase; FET, free exploration test; FMR1, fragile X mental retardation; FNE, forced novelty exploration; Hdc, histidine decarboxylase; KD, knockdown; KI, knock-in; KO, knockout; LD, light/dark test; MB, marble burying; MC, mirror chamber; Mut, mutation; NCAM, neural cell adhesion molecule; NO, novel object; NOS, nitric oxide synthase; NSF, novelty-suppressed feeding; OF, open field; SA, successive alleys; SEP, shock–escape paradigm; SF1, steroidogenic factor 1; SI, social interaction; SIH, stress-induced hyperthermia; ST, staircase test; Tg, transgenic; TRH, thyrotropin releasing hormone; TSC, tuberous sclerosis; VOG, Vogel conflict test.

individuals carrying the S allele of the 5-HTT gene displayed higher levels of trait anxiety than LL homozygotes. This observation was confirmed by several other studies, which demonstrated an association between the S allele and various measures of heightened fear and anxiety in normal populations (Hariri and Holmes, 2006). A functional polymorphism in the promoter region of the human Htr1a gene (coding for the human 5-HT_{1A} receptor) that regulates receptor levels has been shown to be linked to stress-related disorders, such as depression, response to antidepressants, and amygdala reactivity (Fakra et al., 2009; Le François et al., 2008; Lemonde et al., 2003).

In this context, 5-HT_{1A} and 5-HTT knock-out (KO) mice provide a unique means to study the effect of loss of Htr1a and 5-HTT gene function on anxiety-related behaviors under

genetically and environmentally controlled conditions. In 1998, Ramboz and colleagues claimed that mice lacking the 5-HT_{1A} receptor by homologous recombination may represent a valid animal model of anxiety-related disorder since they showed increased emotionality in the elevated plus maze test (Ramboz et al., 1998). This finding was confirmed by several other studies, which demonstrated that KO mice lacking the 5-HT_{1A} receptors display increases in fear-related behaviors in a variety of different experimental procedures, including elevated plus maze, open field, stress-induced hyperthermia, light/dark, and novelty-suppressed feeding tests (Bailey and Toth, 2004; Gleason et al., 2010; Gross et al., 2002; Heisler et al., 1998; Klemenhagen et al., 2005; Parks et al., 1998; Pattij et al., 2002a; Toth and Sibille, 1998; Tsetsenis et al., 2007; see Table 21.1). It is interesting to note that the anxiety-like phenotype was observed in different strains based either on single (i.e., 129/Sv, Swiss, B6, or C57BL/6J) or mixed background, indicating a robust anxiety-like phenotype of 5-HT_{1A} mutant mice. Gain-of-function experiments, in which 5-HT_{1A} receptors were ectopically overexpressed in forebrain areas such as the cortex and striatum, reversed the increased anxiety behavior in 5-HT_{1A} KO mice (Gross et al., 2002), while the loss-of-function approach, where 5-HT_{1A} autoreceptors were selectively suppressed throughout life showed increased anxiety in the adult. Conversely, loss of endogenous heteroreceptors beginning either in the early postnatal period or in adulthood was not sufficient to impact anxiety-like behavior, suggesting that under normal conditions, endogenous 5-HT_{1A} forebrain heteroreceptors are not the primary mediators of 5-HT's effect on developing anxiety circuitry (Richardson-Jones et al., 2011). Pharmacological studies using 5-HT_{1A} receptor KO mice are sparse and were mainly undertaken to confirm the involvement of the 5-HT_{1A} receptor in the anxiolytic-like effects of 5-HT_{1A} agonists, rather than to screen potential anxiolytics. Not surprinsingly in these studies, the 5-HT_{1A} receptor agonists and anxiolytic agents, buspirone and flesinoxan, were inactive in 5-HT_{1A} KO mice (Pattij et al., 2000; Ramboz et al., 1998), confirming the involvement of this receptor in the anxiolytic-like action of these molecules. A few studies showed that the prototypical benzodiazepine (BZ) anxiolytics, diazepam and alprazolam, produced anxiolytic-like effects in 5-HT_{1A} KO mice by attenuating their anxiety-related phenotype (Bailey and Toth, 2004; Pattij et al., 2002a, 2002b).

Initial studies examined the effects of loss-of-function of the 5-HTT and observed increased anxiety in various tests that are validated for their sensitivity to drugs that affect anxiety in humans, such as the elevated plus maze, the open field, the novelty-induced suppression of feeding, and light/dark exploration tests (Holmes et al., 2003c, 2003d; Table 21.1). This anxiety-like phenotype has been replicated in a separately generated 5-HTT KO mouse model, and a study using a third line of mutants lacking the C-terminus of the 5-HTT also revealed heightened anxiety-like behavior in 5-HTT KO mice (Ansorge et al., 2004; Heiming et al., 2009; Line et al., 2011; Lira et al., 2003; Murphy and Lesch, 2008; Wellman et al., 2007; Zhao et al., 2006). As is the case with the 5-HT_{1A} receptor, the effects of genetic inactivation of the 5-HTT on anxiety-like behavior in mice are robust and provide an independent line of evidence supporting a link between the low-expressing 5-HTT gene variant with anxiety in humans. However, despite compelling evidence that 5-HTT KO mice may represent a valid model of anxiety, no clinically effective anxiolytic drugs have been tested in these animals.

In contrast to the robust anxiety-like phenotype seen in 5-HT_{1A} and 5-HTT mutant mice, there are a few, sometimes unconvincing or anecdoctal, reports on increased anxiety-related behaviors following the deletion of other 5-HT receptors or elimination proteins (Table 21.1). There is, for example, evidence of an anxiety-like phenotype in 5-HT_{2C} receptor KO mice in the emergence test (Tecott et al., 1998). However, performance in this test is strongly influenced by alterations in spontaneous locomotor activity, suggesting that the behaviors displayed by 5-HT_{2C} KO mice were unrelated to anxiety.

In summary, both 5-HT_{1A} and 5-HTT KO mice appear to be valid models of anxiety. In this context, it is interesting to note that the anxiety-like behavior in the 5-HTT mice can be normalized by the 5-HT_{1A} antagonist WAY 100635, suggesting that the postsynaptic 5-HT_{1A} receptor is a participant in these anxiety-like behaviors (Holmes et al., 2003d). Moreover, transient pharmacological inhibition of 5-HTT by the SSRI fluoxetine during early development has been shown to mimic the anxiety phenotype of 5-HTT mutant mice, suggesting that a developmental mechanism explains how low 5-HTT function increases vulnerability to anxiety disorders (Ansorge et al., 2004).

Genetic models of anxiety based on manipulations of the GABA system

It is widely acknowledged that γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the brain is implicated in the pathophysiology of several psychiatric disorders, including anxiety and depression (Brambilla et al., 2003). There are two classes of GABA receptors: ionotropic $GABA_A$ receptors and metabotropic $GABA_B$ receptors. Whereas $GABA_A$ receptors are made up of some 20 protein subunits (six different α subunits, three β, three γ, and several other subunits, which are generally less abundantly expressed: δ, π, θ, and ε; Barnard et al., 1998), the composition of which determines the function of the receptor complex, $GABA_B$ receptors are heterodimers made up of two subunits, $GABA_{B(1)}$ and $GABA_{B(2)}$, both necessary for $GABA_B$ receptors to be functionally active (Calver et al., 2002). Benzodiazepines and compounds with known anxiolytic properties, such as barbiturates, ethanol, and neuroactive steroids, exert their effects via an action at the $GABA_A$ receptor (Haefely, 1983; Sieghart, 1995). Although less compelling than for $GABA_A$ receptors, there is evidence indicating that $GABA_B$ receptors play a role in anxiety (Cryan and Kaupmann, 2005). In an attempt to better understand the functional roles of $GABA_A$ and $GABA_B$ subunits, mice with genetic alterations at the level

of individual $GABA_A$ subunits have been generated. As will be shown below, several of these mutant lines have been claimed to represent models of chronic or trait anxiety.

The γ2 subunit of $GABA_A$ receptors is highly expressed throughout the developing and adult brain, and is essential for the formation of the majority of BZ binding sites, normal $GABA_A$ receptor channel conductance, and synaptic clustering of $GABA_A$ receptors at postsynaptic sites (De Blas, 1996; Essrich et al., 1998; Whiting, 1999). Crestani and colleagues were the first to report that mice that are heterozygous for the γ2 subunit ($\gamma2^{+/-}$; most mice homozygous for the mutation died within days of birth) exhibit increased anxiety-like behavior in a variety of experimental procedures, involving both spontenaous anxiety-like behavior and learned fear responses (Table 21.1; Crestani et al., 1999). In particular, $\gamma2^{+/-}$ mice exhibited increased risk assessment and neophobic behaviors in a free-choice exploration test that is devoid of intrinsic stress and marked avoidance to ambiguous stimulus in a cued and contextual fear conditioning paradigm. This enhanced emotional behavior of $\gamma2^{+/-}$ mice was attenuated by the administration of the BZ diazepam, an effect that fits well with the observation of increased sensitivity to BZs seen in anxious patients (O'Boyle et al., 1986). The finding of increased anxiety in $\gamma2^{+/-}$ mice has since been replicated by the same group, and also others (Chandra et al., 2005; Earnheart et al., 2007; Homanics et al., 1999). In one of these studies (Earnheart et al., 2007), even a modest and region-specific decrease in γ2 subunit-containing $GABA_A$ receptors induced in precursors of glutamatergic neurons of the embryo and extending to adult neural progenitor cells resulted in a pronounced increase in emotionality, suggesting that reduced γ2 function during development can serve as a common molecular substrate for anxiety behaviors. Reports on increased anxiety-related behaviors following the deletion of other $GABA_A$ receptor subunits are sparse. To the best of our knowledge there are only three studies that have reported that mice with targeted ablation of the genes encoding the α1, α2, or the β3 subunit of the $GABA_A$ receptors have enhanced anxiety-like behaviors under specific experimental conditions (Dixon et al., 2008; Hashemi et al., 2007; Table 21.1).

The strongest evidence to date from studies in animals for a role of $GABA_B$ receptors in anxiety was demonstrated by the phenotype of $GABA_B$ receptor-deficient mice. Targeted deletion of either the $GABA_{B(1)}$ or $GABA_{B(2)}$ receptor subunits in mice resulted in a complete loss of $GABA_B$ functions accompanied by an anxious phenotype in a variety of paradigms, involving mainly exploratory-based behaviors (Mombereau et al., 2004, 2004b, 2005; Table 21.1). The $GABA_{B(1)}$ subunit is predominantly expressed as one of two isoforms: $GABA_{B(1a)}$ or $GABA_{B(1b)}$ (Steiger et al., 2004). In an attempt to dissect the physiological roles of these isoforms, mice deficient in either the $GABA_{B(1a)}$ or $GABA_{B(1b)}$ isoforms have been generated. However, the results of evaluation of these mutants in anxiety tests revealed only modest increase in anxiety-related behaviors as compared to $GABA_{B(1)}$ or $GABA_{B(2)}$ KO mice (Jacobson et al., 2007).

GABA is generated in the brain by the enzyme glutamic acid decarboxylase (GAD), which exists in two isoforms, GAD65 and GAD67 (Martin and Rimvall, 1993). With the development of null mutant mice for GAD65 and GAD67 it has become possible to investigate the functional relevance of these isozymes and their contribution to specific GABA-mediated neural functions. While GAD67 mutant mice die shortly after birth, GAD65 ($Gad65^{-/-}$) null mutant mice are viable (Asada et al., 1996, 1997). These latter showed increased emotionality in several tests involving both spontenaous anxiety-like behavior and learned fear responses (Bergado-Acosta et al., 2008; Kash et al., 1999; Sangha et al., 2009; Stork et al., 2000, 2003). In particular, $Gad65^{-/-}$ mice exhibited increased avoidance behaviors of aversive places in the elevated zero maze and open field, and marked avoidance was observed during both cued and contextual fear conditioning. These effects were attributed to the decreased levels of GABA rather than to changes in postsynaptic $GABA_A$ receptor density in these mice as demonstrated notably by radioligand receptor binding results, which revealed no changes in these sites (Kash et al., 1999). Moreover, $Gad65^{-/-}$ mice displayed a diminished response to the anxiolytics diazepam and pentobarbital, effects explained by a direct consequence of the lack of GAD65-generated GABA without a modulation by postsynaptic events (Kash et al., 1999).

Taken as a whole the findings on the effects of genetic manipulation of the GABA system have provided less convincing evidence, as compared to the 5-HT system, that mutant mice of the former system may represent valid models of anxiety disorders. This is particularly true for mice bearing mutations of $GABA_A$ or $GABA_B$ receptor subunits, with the exception perhaps of the γ2 subunit of the $GABA_A$ receptor. Regarding mutations of the GAD isoforms, it would have been interesting to study the phenotype of heterozygote mice of GAD67 as was done for the γ2 subunit of $GABA_A$ receptors, but no such data are available.

Genetic models of anxiety based on manipulations of neuropeptide systems

In the past decades, there has been increasing interest and, consequently, active and dynamic research on neuropeptides (for a recent review see Griebel and Holsboer, 2012). Neuropeptides are attractive therapeutic targets for anxiety disorders (Holmes et al., 2003a). They are short-chain amino acid neurotransmitters and neuromodulators, often localized in brain regions that mediate emotional behaviors and the response to stress (Belzung et al., 2006). Progress in identifying the role of neuropeptides in stress has been facilitated by recent developments in screening for selective small-molecule neuropeptide ligands that cross the blood–brain barrier. Rodents with mutations in genes encoding neuropeptides and their receptors have been developed for nearly all targets of interest for anxiety disorders. While there are sparse reports on anxiogenic-like

phenotypes in mice deficient in or overexpressing galanin GAL-R1 receptor, neuropeptide Y, or its Y1 and Y2 receptors, cholecystokinin CCK2 receptor, nociceptin or nociceptin receptor, angiotensin II receptor, vasopressin V_{1a} receptor, enkephalin, or thyrotropin releasing hormone, the most compelling evidence comes from studies that investigated targeted mutation of the corticotropin-releasing factor (CRF) system (Table 21.1).

The 41 amino acid neuropeptide CRF, which is well known for its crucial role in orchestrating the hypothalamic–pituitary–adrenal (HPA) axis response to stress, has been the subject of intense investigation in the pathophysiology and treatment of anxiety disorders (Griebel and Holsboer, 2012). CRF is synthesized in neurons of the paraventricular hypothalamic nucleus and released into the pituitary portal blood where it triggers the secretion of adrenocorticotropin (ACTH) from the anterior lobe. Subsequently, corticosterone (in rodents) or cortisol (in humans) is secreted from the adrenal cortex into the blood and exerts a negative feedback on the HPA axis. CRF and its two G-protein-coupled CRF receptor subtypes (CRF-R1 and CRF-R2) are widely distributed throughout the brain. In addition, the biological activity of CRF is influenced by CRF-binding protein. This glycoprotein is highly expressed in many tissues including the brain, where it binds to CRF with high affinity, thus competing with receptor binding and subsequent signaling (for a review on the CRF system see Steckler and Dautzenberg, 2006).

Genetic manipulations of the CRF system include deletion of CRF-R1 or CRF-R2, or ablation of the CRF-binding protein, and ovexpression of the neuropeptide CRF (Table 21.1). CRF-R1 null mutant mice have been reported to display reduced anxiety-like behaviors (Gammie and Stevenson, 2006; Kresse et al., 1998; Muller et al., 2003; Refojo et al., 2011; Smith et al., 1998), findings that are compatible with the anxiolytic-like effects of CRF-R1 antagonists in preclinical studies (Griebel, 1999; Griebel and Holsboer, 2012). Interestingly, while CRF-R1 deletion in forebrain glutamatergic circuits was found to reduce anxiety, selective deletion of CRF-R1 in midbrain dopaminergic neurons increased anxiety-like behavior, suggesting a bidirectional role of CRF-R1 in anxiety and that an imbalance between CRF-R1-controlled anxiogenic glutamatergic and anxiolytic dopaminergic systems might lead to emotional disorders (Refojo et al., 2011).

CRF-R2 and CRF-binding protein KO, as well as animals overexpressing CRF, showed a clear anxiogenic-like phenotype (Table 21.1). Although initially different groups, using independently generated line of CRF-R2 receptor mutant mice, demonstrated comparable elevated anxiety-related behaviors in CRF-R2-deficient animals using exploration-based models of anxiety (Bale et al., 2000; Coste et al., 2000; Kishimoto et al., 2000), more recent experiments using the startle reflex as an index of anxiety were unable to confirm the earlier findings (Risbrough et al., 2009). Moreover, this phenotype is difficult to reconcile with the anxiolytic-like effects observed in numerous studies using peptide CRF-R2 antagonists or antisense knockdown of CRF-R2 (Hammack et al., 2003; Heinrichs et al., 1997a; Ho et al., 2001; Liebsch et al., 1999; Pelleymounter et al., 2002; Radulovic et al., 1999; Sananbenesi et al., 2003; Takahashi et al., 2001). Additional research would be needed to further define the role of CRF-R2 receptors in anxiety behaviors. However, the finding that blockade of the CRF-R2 receptor could have negative effects on cardiovascular function has dramatically reduced research efforts on this target within the last decade. As indicated above, transgenic mice conditionally overexpressing CRF have been consistently reported to display a consistent anxiety-like phenotype and elevation in corticosterone level (Karolyi et al., 1999; Kolber et al., 2010; Lu et al., 2008; Ramesh et al., 1998; Stenzel-Poore et al., 1996), which fits well with the numerous findings showing that central infusion of CRF produced anxiogenic-like effects and stimulates corticosterone release (Griebel, 1999). Altogether, these studies participated greatly in the demonstration of the importance of the CRF system for controlling anxious behavior.

Caveats in genetic models of anxiety disorders

These genetic animal models of anxiety have at first glance clear advantages over classical anxiety models in which baseline levels of anxiety of a "normal" subject are increased artificially by exposure to aversive stimuli. They may provide a unique opportunity to study human anxiety and emotional disorders. However, all these genetic models are based on the deletion of a single gene, and it is widely acknowledged that the modulation of anxiety processes involves multiple genes. It is clear that any behavioral phenotype observed in a gene mutant mouse will be the product of a complex, epistatic interaction between the mutation and the genetic background on which it is placed. Therefore, it can hardly be claimed that mice with targeted mutation represent models of "general" anxiety disorders, and it would be unreasonable to use them for the screening of potential novel anxiolytics acting at a target unrelated to the neurotransmitter system of the mutation. It is also important to note that mutant mice studies use DNA constructs and embryonic stem cells invariably derived from 129 substrains (e.g., 129/SvJ, 129/SvEv, 129/Ola), later mixed with a separate inbred strain (often C57BL/6). Strain differences in emotionality have repeatedly been reported (Griebel et al., 2000). As a striking example, there are marked differences in anxiety-related behaviors between C57BL/6 and 129 substrains and the outcome of a study using mutant mice may be dependent upon which 129 substrain is tested (Holmes, 2001). Moreover, it is noteworthy that most of the anxious phenotypes of mutant mice were observed in a limited number of anxiety assays, mainly based on avoidance and exploration behaviors (see column 3 of Table 21.1). Therefore, it is not clear whether these mice can be considered as models of "certain" aspects of anxiety or "global" anxiety. There is no "gold standard" among the anxiety tests. Ideally, the anxious phenotype should be elicited across tests that involve different aspects of the anxiety repertoire.

Alterations across the entire behavioral repertoire following genetic intervention can often confound the analysis of anxiety behavior. It is vital that such confounding behaviors are taken into account, thus avoiding erroneous interpretations of behavioral data. A thorough determination of any confounding abnormalities present in genetically modified animals prior to behavioral testing is strongly recommended. Other factors that can influence behavior in anxiety tests, such as early life experience, previous test exposure, and compensatory changes, have been discussed elsewhere and readers are referred to these publications for further details (Belzung et al., 2008; Belzung and Griebel, 2001; Cryan and Holmes, 2005; Jacobson and Cryan, 2010).

Conclusion

Development of genetic animal models has proven invaluable in the dissection of the neurobiological basis of anxiety behavior and in indicating potential therapeutic avenues for treatment of anxiety disorders. The issue of genetic background and other problems more associated with the behavioral methodology highlight some of the caveats that are essential to consider when using genetically manipulated mice. Combining these genetic animal models with endophenotype-based and translationally valid models of anxiety should represent a central strategy in future research efforts for developing novel treatments for anxiety disorders.

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