GENETIC APPROACHES TO THE STUDY OF ANXIETY

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■ **Abstract**   Anxiety and its disorders have long been known to be familial. Recently, genetic approaches have been used to clarify the role of heredity in the development of anxiety and to probe its neurobiological underpinnings. Twin studies have shown that a significant proportion of the liability to develop any given anxiety disorder is due to genetic factors. Ongoing efforts to map anxiety-related loci in both animals and humans are underway with limited success to date. Animal models have played a large role in furthering our understanding of the genetic basis of anxiety, demonstrating that the genetic factors underlying anxiety are complex and varied. Recent advances in molecular genetic techniques have allowed increasing specificity in the manipulation of gene expression within the central nervous system of the mouse. With this increasing specificity has come the ability to ask and answer precise questions about the mechanisms of anxiety and its treatment.

ANXIETY AND ANXIETY DISORDERS

Wariness of threatening aspects of the environment is a protective, if at times uncomfortable, trait. In humans the experience of such wariness is called anxiety, and it is typically accompanied by characteristic autonomic responses and defensive behaviors. Whereas anxiety in many settings is protective, excessive anxiety can prove disabling. Individuals who suffer from anxiety disorders are often crippled by overwhelming emotional and physical symptoms. As many as 25% of adults will, at one point in their lives, suffer from one of the six described forms of anxiety disorder (Kessler & Greenberg 2002): panic disorder, characterized by unpredictable, rapid-fire attacks of intense anxiety; generalized anxiety disorder, marked by excessive worry in multiple areas; social anxiety disorder, characterized by fear and avoidance of social situations; specific phobia, notable for intense fear of a specific trigger; posttraumatic stress disorder, characterized by intrusive, anxiety-provoking memories of trauma; and obsessive-compulsive disorder, marked by anxious obsessions and anxiety-reducing compulsive behaviors. In aggregate these disorders account for tremendous morbidity, and the collective economic cost of these disorders is estimated to be over $40 billion per year

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(Kessler & Greenberg 2002). Although effective treatments for anxiety disorders are available, many patients are left with residual symptoms or experience side effects that limit their adherence to prescribed regimens (Gorman et al. 2002). Improved understanding of the neural mechanisms of anxiety and its treatments, therefore, could reduce the individual and societal costs of anxiety disorders by pointing the way toward improved treatments.

In order to attempt an understanding of neural mechanisms, numerous investigators have turned to animal models of anxiety. Like humans, animals respond to the potential presence of a threat with characteristic autonomic responses and defensive behaviors. These physiological and behavioral parameters can be measured with relative ease and have been validated with drugs known to be anxiolytic or anxiogenic in humans. This approach has been used to characterize numerous models of anxiety-like behavior in laboratory animals (Borsini et al. 2002). Paradigms such as fear-conditioned freezing and fear-potentiated startle rely on learned associations between innocuous and painful stimuli; pairing of, say, a particular sound with a mild shock causes the animal to display anxiety-like defensive behaviors such as freezing (periods of attentive stillness) or enhanced startle (heightened reactivity to a loud, sudden noise) in response to later presentations of the sound (Davis et al. 2003, LeDoux 2000). Other animal models of anxiety depend upon innate, species-specific danger signals. The elevated plus maze and novelty-suppressed feeding tasks, for example, measure a rodent's avoidance of exposed or novel areas, in which they might be more vulnerable to predation (Rodgers & Dalvi 1997, Santarelli et al. 2003). Anxiolytic drugs, such as benzodiazepines, reduce the expression of anxiety-like behaviors in each of these paradigms (Conti et al. 1990, Pellow & File 1986, Shephard & Broadhurst 1982).

Pharmacological and neurobiological analysis of anxiety disorders and animal models of anxiety have helped to identify neurotransmitters and neural systems involved in the expression of anxiety. The most widely prescribed classes of anxiolytic drugs, the benzodiazepines and the selective serotonin reuptake inhibitors (SSRIs), modulate γ-aminobutyric acid (GABA)- and serotonin-mediated neurotransmission, respectively (Gorman et al. 2002). Likewise, injection of drugs that alter GABAergic or serotonergic neurotransmission alter anxiety-like behavior in animals (Borsini et al. 2002, Griebel 1995). Pharmacological challenge studies in anxiety disorder patients have also implicated the noradrenergic system, especially in panic disorder (Sullivan et al. 1999). Studies of stress physiology in both animals and humans have suggested a powerful role for the adrenocortical system and its neuropeptide regulator, corticotrophin-releasing hormone (CRH), in anxiety disorders and anxiety-like behavior (Clark & Kaiyala 2003). Imaging studies in humans, as well as lesion and microinjection studies in animals, have in turn implicated various brain structures in anxiety, including the amygdala, the hippocampus, the cingulate cortex, the hypothalamus, and various brainstem areas (Charney & Drevets 2002). Hypothetical anxiety circuits have been proposed to explain particular forms of anxiety-like behavior, such as those suggested by Davis et al. (2003) and LeDoux (2000), to underlie the learning and expression of conditioned-fear behaviors (see Figure 1).
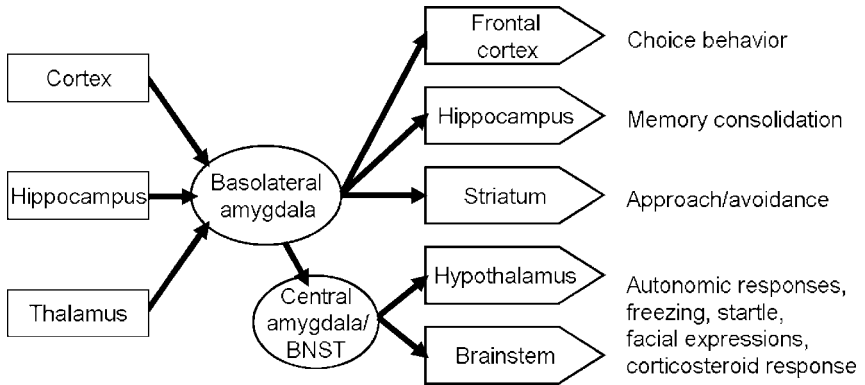
Figure 1 Schematic diagram of a proposed fear circuit. Adapted from Davis et al. (2003).

It has long been known that anxiety disorders, like most psychiatric illnesses, have important heritable components. Hopes that linkage studies might reveal single genes predisposing for specific disorders have gone unfulfilled thus far (Finn et al. 2003). Single-gene knockout studies, on the other hand, have led to the identification of numerous genes seemingly required for normal anxiety-like behavior in mouse models (Finn et al. 2003, Lesch 2001b). Indeed, the diversity of single-gene deletions that result in anxiety phenotypes in animals is so great that it calls into question the specificity of these manipulations. Here we examine the impact of recent forays into genetic approaches to anxiety. We highlight particular studies that best illustrate the capabilities and, in some cases, limitations of particular genetic approaches. These results suggest areas of focus for future use of genetic technology and point out that harnessing the full power of genetics will likely require applying more traditional neuroscience techniques in order to fully characterize the impact of genetic manipulations.

GENETIC APPROACHES TO HUMAN ANXIETY DISORDERS

Numerous studies have demonstrated that anxiety disorders run in families (Fyer et al. 1995, Merikangas & Pine 2002, Noyes et al. 1987). Individuals with first-degree relatives who suffer from a given anxiety disorder are 4–6 times more likely to develop that disorder than individuals without such relatives (Hettema et al. 2001a). Twin studies have since confirmed that the great majority of this familial risk is due to genetic heritability. Comparisons of concordance rates in dizygotic and monozygotic twins have established that 30%–50% of the individual variability in risk to develop a given anxiety disorder is due to genetic factors (Hettema et al. 2001b, Kendler 2001, Roy-Byrne et al. 2002, Skre et al. 2000). The remainder of this variability is accounted for by environmental factors specific to the individual

twin, as opposed to environmental experiences shared within the family (Kendler 2001). These data demonstrate that anxiety disorders have a moderate genetic predisposition strongly influenced by environmental interactions.

The nature of this moderate predisposition is not entirely clear, however. Twin studies have consistently demonstrated a genetic predisposition that accounts for 30%–35% of the liability to develop posttraumatic stress disorder (PTSD) following exposure to traumatic events (Goldberg et al. 1990, True et al. 1993). Intriguingly, there is also a genetic predisposition for exposure to certain types of trauma; thus, genetic factors account for 20% and 35% of the liability to exposure to assaultative and combat-related trauma, respectively (Lyons et al. 1993, Stein et al. 2002). With regard to civilian assaults, a high correlation between genetic effects on exposure to trauma and development of PTSD symptoms suggest that a single genetic factor increases the likelihood of both exposure and pathological reaction to extreme trauma (Stein et al. 2002). These data point out that the straightforward interpretation of genetic factors as grounded in strictly neurobiological mechanisms ignores the fact that genetic factors influence how individuals interact with their environment. Indeed, Kendler (2001) has suggested that up to 20% of the genetic influences in psychiatric disorders could be mediated by such indirect mechanisms.

Attempts to map the loci underlying these genetic factors using genome-wide linkage scans have shown scattered promise. Panic disorder has been the focus of most of the genetic studies because of its relatively distinctive symptomatology. Findings to date have been mostly weak and inconclusive (Crowe et al. 2001, Knowles et al. 1998, Weissman 1993), although recently panic disorder has been tentatively linked with sites on 7p (Logue et al. 2003) and 9q (Thorgeirsson et al. 2003). Attempts to take advantage of possible genetic syndromes involving cotransmission of panic disorder and joint laxity or kidney/bladder disease have suggested that specific subtypes of panic might be more amenable to traditional mapping techniques (Gratacos et al. 2001, Hamilton et al. 2003, Weissman et al. 2000), but these studies have yet to be replicated. The data for other anxiety disorders are sparse. One recent study revealed a model-dependent link to a single marker on chromosome 14 in simple phobia (Gelernter et al. 2003). An initial genome-wide scan for linkage to obsessive compulsive disorder failed to reveal any significant associations (Hanna et al. 2002).

Candidate Genes and Gene-Environment Interactions

Numerous attempts have been made to link the genetic predisposition toward anxiety disorders with specific candidate genes (for a recent review, see Finn et al. 2003). Taking their cues from effective treatments for anxiety, investigators have targeted genes encoding components of the monoaminergic and GABAergic systems. Whereas a genetic association between the gene encoding for the serotonin transporter (SERT) and anxiety traits has been found (see below), no such association was found with panic disorder or obsessive-compulsive disorder (Frisch

et al. 2000, Hamilton et al. 1999). A longer, more active form of the monoamine oxidase A promoter has been found with higher frequency in female panic disorder patients than in normal controls in one study (Deckert et al. 1999), but this result did not survive replication (Hamilton et al. 2000). Similarly, contradictory data have been reported for catecholamine-O-methyltransferase in obsessive-compulsive disorder and panic disorder (Alsobrook et al. 2002, Hamilton et al. 2002, Karayiorgou et al. 1999, Ohara et al. 1998) and the serotonin 1B (5-HT1B) receptor in obsessive-compulsive disorder (Di Bella et al. 2002, Mundo et al. 2000). The gene encoding the GABA-synthetic enzyme GAD65 has been associated with the panic disorder–related trait of behavioral inhibition in children (Smoller et al. 2001b), but none of the eight genes encoding GABA receptors tested thus far has been linked to panic disorder (Finn & Smoller 2001, van den Heuvel et al. 2000). Several other attempts to link various other specific genes with specific anxiety disorders have been mostly negative (Finn & Smoller 2001, Finn et al. 2003).

Several studies, but not all, have found a link between anxiety traits and a variation in the gene encoding the SERT (Greenberg et al. 1999, Gustavsson et al. 1999, Jorm et al. 1998, Lesch 2001a, Lesch et al. 1996). A simple repeat sequence lies within the SERT gene promoter. There are two different repeat lengths in the caucasian population: a 14 repeat short (*s*) allele and a 16 repeat long (*l*) allele (Lesch et al. 1996). Adults who are homozygous for the *s* allele have lower levels of SERT activity and score higher for "neuroticism" on an anxiety test battery than heterozygous *s/l* or homozygous *l/l* adults (Greenberg et al. 1999, Lesch et al. 1996). Infants homozygous for the *s* allele have similar increases in anxiety-related measures (Auerbach et al. 1999). Intriguingly, recent functional imaging studies have found that adults homozygous for the *s* allele have greater amygdala activation during the observation of fearful faces (Hariri et al. 2002). Nonetheless, the effect of the *s* allele on trait anxiety is modest at best, accounting for approximately 4% of the phenotypic variance (Lesch et al. 1996).

The low predictive value of the SERT polymorphism genotype allows for multiple interacting mechanisms, including, of course, additional genes. Recent research supports the possibility that gene/environment interactions account for a portion of the additional variance. In humans, the *s* allele increases the risk of major depressive disorder but only in those individuals with a previous history of major life stressors and/or childhood trauma (Caspi et al. 2003). In monkeys, as in humans, short and long versions of the SERT promoter repeat exist (Lesch et al. 1997). Also in monkeys, the *s* allele is associated with an increase in the serotonin metabolite, 5-HIAA (consistent with decreased SERT activity), and an increase in emotional reactivity (Champoux et al. 2002). These effects of the SERT polymorphism in monkeys are dependent on early rearing environment. Monkeys reared by their mothers show normal levels of 5-HIAA regardless of SERT genotype, whereas nursery-reared monkeys have increased 5-HIAA if they carry the *l/s* genotype, but not if they carry the *l/l* allele (Bennett et al. 2002). An identical rearing by genotype interaction was seen in an anxiety-related behavioral variable (Champoux et al. 2002). These data demonstrate that physiological and behavioral effects of the

SERT polymorphism are dependent on a permissive rearing environment in both humans and nonhuman primates. They provide a clear example of an interaction between genes and environment in the regulation of anxiety-related behaviors.

ANIMAL MODELS OF ANXIETY

Whereas human studies have established the genetic basis of anxiety, animal studies have been used to attempt to further clarify its genetic determinants. There are numerous animal models of anxiety-like behavior. The relevance of these models to human anxiety disorders is derived first and foremost from pharmacological validity. Benzodiazepines, the archetypal anxiolytic drugs, reduce the level of anxiety-like behavior in virtually every animal model of anxiety (Borsini et al. 2002). The development of the SSRIs as the preferred pharmacological treatment for anxiety has called into question the direct relevance of many of these models to specific anxiety disorders, as the effects of SSRIs and other nonbenzodiazepine anxiolytics on these models are at best inconsistent (Borsini et al. 2002). Nonetheless many of these models have been useful when combined with genetic approaches to the study of anxiety.

Fear Versus Anxiety: Learned Fear Paradigms

As models for anxiety disorders are developed and refined, a key issue that arises is the difference between fear and anxiety. Davis (1998) has suggested that fear is the response to a specific, stimulus-linked threat, whereas anxiety is a nonstimulus-linked defensive state. Gray & McNaughton (2000) similarly proposed that fear is the defensive response to the actual presence of a threat, whereas anxiety is the defensive response to the potential presence of a threat. These two definitions both characterize fear as tied to specific stimuli and anxiety as more generally linked to situations or environments. Both may have their parallels in human anxiety disorders. Specific phobia and PTSD, for example, are disorders in which emotional and behavioral responses are often tied to specific stimuli, such as snake phobias, or flashbacks triggered by loud noises. Patients with generalized anxiety disorder and panic disorder, on the other hand, experience symptoms without regard to the presence of particular stimuli.
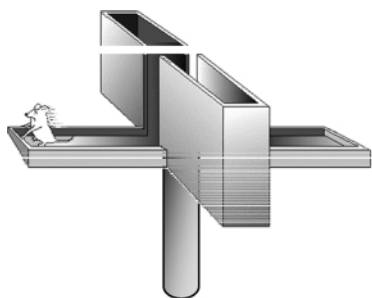
Animal models of stimulus-linked defensive behaviors have been extensively studied, especially with regard to the mechanisms by which stimuli and responses are associated. Fear-conditioned freezing and fear-potentiated startle involve pairing of a stimulus (typically a tone or a specific context) with shock. The learned association between the stimulus and the shock then results in freezing behavior or an increased startle response. Benzodiazepines decrease the time spent freezing after presentation of a footshock (Conti et al. 1990) or after exposure to a conditioned stimulus (Beck & Fibiger 1995, Pletnikov et al. 1996, Quartermain et al. 1993). The conditioned stimulus-induced potentiation of the startle response is also

decreased by benzodiazepines (Davis 1979). Acute treatment with SSRIs has been reported to inhibit both the learning and expression of conditioned fear responses (Inoue et al. 1996). Although chronic treatment with the SSRI fluvoxamine has been reported to have no effect on conditioned freezing, the duration of treatment (15 d) may have been inadequate (Li et al. 2001).
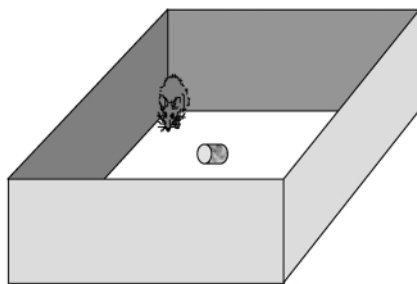
Genetic approaches to learned fear have received considerable attention, most prominently with regard to genetically manipulated mice. Numerous knockouts have been shown to have altered fear conditioning, although most studies focus on understanding the learning component itself (Ammassari-Teule et al. 2001, Chen et al. 1994, Crestani et al. 2002, Frankland et al. 2001, Howe et al. 2002, Pape & Stork 2003, Stork et al. 2003, Wei et al. 2002). Differences in inbred and selectively bred strains have been characterized (Balogh & Wehner 2003, Bolivar et al. 2001, Falls et al. 1997, Nie & Abel 2001, Paterson et al. 2001), and attempts have been made to map genetic loci that modify fear-conditioning behaviors (Fernandez-Teruel et al. 2002, Radcliffe et al. 2000, Wehner et al. 1997). Fear conditioning can be readily performed in humans, and a recent twin study suggested that genetic factors account for approximately 35%–45% of the variance in a nonclinical population (Hettema et al. 2003). This proportion is within the range of those described for anxiety disorders (see above). Attempts to characterize fear-potentiated startle in clinical populations have thus far yielded only subtle differences between patients and controls (Grillon et al. 1994, Morgan et al. 1995).

Models of Innate Anxiety

Much of the use of genetic approaches to study anxiety have focused on tests of innate anxiety, rather than fear learning. Perhaps the best characterized model of innate anxiety is the elevated plus maze. The maze consists of an elevated, plus-shaped platform, two arms of which are enclosed by high walls (Figure 2). The other two arms are unenclosed or surrounded by a small lip. Consistent with the idea

Elevated plus maze   Novelty-suppressed feeding

Figure 2   Animal models of innate anxiety.

that the open arms provoke an anxiety-like behavioral state, rats and mice exposed
to the maze will make fewer entries into and spend less time in the open arms than
the closed arms (Lister 1987, Pellow et al. 1985). The validity of these measures
as a model of anxiety has been confirmed pharmacologically, ethologically, and
physiologically. Benzodiazepines increase the number of open-arm entries and
time spent in the open arms, whereas yohimbine and other anxiogenic agents
decrease these parameters (Lister 1987, Pellow et al. 1985, Pellow & File 1986).
Ethological analysis has demonstrated that animals perform more defensive and
anxiety-related behaviors in the open arms than in the closed arms (Cruz et al.
1994, Pellow et al. 1985, Rodgers & Johnson 1995). Animals confined to the open
arms have higher plasma corticosterone concentrations and produce more fecal
boli than those confined to the closed arms (Pellow et al. 1985). A common variant
of the elevated plus maze is the elevated zero maze, in which a circular platform
is divided into four quadrants, two of which are enclosed and two of which are
open, eliminating the potentially problematic center of the elevated plus (Shepherd
et al. 1994). Several other tests rely on relatively "safe" and "unsafe" aspects of
the testing environment, including the open field (in which the rodents prefer the
periphery to the center) and light/dark test (in which rodents prefer the dark area).
These tests are also generally responsive to benzodiazepine anxiolytics (Borsini
et al. 2002).

The novelty-suppressed feeding task is a newer test that depends on the sup-
pression of feeding induced by a novel environment (Figure 2). Animals are food-
deprived and placed in a novel chamber; the latency to eat from a pellet of food
placed in the center is among the parameters measured. Benzodiazepines reduce
this latency (Bodnoff et al. 1989, Shephard & Broadhurst 1982), as does chronic
(but not acute) treatment with SSRIs (Bodnoff et al. 1988, 1989; Merali et al. 2003;
Santarelli et al. 2003). Novelty-suppressed feeding is, therefore, one of the few
tests of anxiety that mimics the time course of anxiolytic efficacy seen in humans.

Interstrain Differences in Anxiety-Like Behaviors

Initial attempts to explore the genetic basis of anxiety with animal models focused
on examining differences in anxiety behaviors between different inbred rodent
strains. It has been clearly established that genetically different strains of mice and
rats behave differently in a variety of tests for anxiety, including those mentioned
above (Avgustinovich et al. 2000, Cook et al. 2001, Griebel et al. 2000, Rex et al.
1996, Tarantino et al. 2000, Trullas & Skolnick 1993, van Gaalen & Steckler 2000);
there are also strain-dependent differences in the responses to anxiolytics in these
tests (Crawley & Davis 1982, Griebel et al. 2000). The relative order of mouse
strains, in terms of level of anxiety-like behaviors, differs depending on the task.
For example, Griebel et al. (2000) found that mice of the NZB strain were among
the most anxious in the elevated plus maze and the least anxious in the light/dark
test. Although some studies suggest anxiety-like behavior in certain tests is corre-
lated [for example, novelty-suppressed feeding and elevated plus behavior in eight

strains of mice (Trullas & Skolnick 1993)], most suggest that different behavioral tests measure different aspects of anxiety (Avgustinovich et al. 2000, Griebel et al. 2000, van Gaalen & Steckler 2000). In rats, a factor analysis of elevated plus and open field behavior in an F1 intercross between two rat strains suggested that anxiety-related behaviors in these two tests loaded onto separate factors (Ramos et al. 1998). A six-strain, four test-factor analysis in rats places anxiety-related behaviors on two factors and locomotion on a third (Ramos et al. 1997). More extensive multitest and multistrain analyses have yet to be implemented.

A recent study of the behavior of several strains in the elevated zero test suggest one potential source of interstrain differences. Cook et al. (2001) found that three inbred mouse strains (C3H, CBA, and FVB) that carry a mutation (*rd1*) leading to retinal degeneration demonstrate uncharacteristically low levels of anxiety-like behavior in this test. For one of these strains (C3H), a congenic strain with the wild-type allele at this locus had elevated levels of anxiety-like behavior compared to the original strain, arguing strongly that the *rd1*-induced visual deficits account for the elevated anxiety levels in the original strain. Because this study used only this one test of anxiety, it is not clear, nor is it likely, that visual deficits can explain the inconsistent strain-dependent results across multiple anxiety models. Nonetheless, this study makes explicit the notion that animal models involve many behaviors and systems other than those directly involved in the expression of anxiety.

As an alternative to relying on undirected inbreeding to establish genetically based variability in anxiety measures, several investigators have used directed breeding to purposefully derive lines of mice or rats expressing high or low levels of anxiety-related behaviors (Broadhurst 1975, Goto et al. 1993, Landgraf & Wigger 2002, Liebsch et al. 1998a, Liebsch et al. 1998b, Suaudeau et al. 2000). Some of these models suffer from the same inconsistencies across anxiety-related tasks as do inbred lines (Broadhurst 1975, Chaouloff et al. 1994). Interestingly, one group has reported selectively breeding rat lines with high (HA) and low (LA) levels of anxiety-like behavior in the elevated plus maze (Liebsch et al. 1998a,b). Further characterization of these lines revealed that HA rats exhibit higher levels of anxiety across several different tasks (Landgraf & Wigger 2002, Liebsch et al. 1998a). Yet the inbred strain and factor analysis studies listed above suggest that different factors govern performance in the different anxiety models. How does one explain that a line of rats bred for behavior on a single anxiety-related task simultaneously inherits multiple seemingly independent anxiety-predisposing factors? Comparing factor analyses of HA, LA, and control strains across different tests, Ohl et al. (2001) found that in the HA and LA rats, anxiety-related factors overwhelmed other factors, explaining a greater proportion of the variance as compared to control rats. These data argue that the genetic predisposition toward increased anxiety in the elevated plus maze inherited by the HA rats is actually a generalized predisposition, trumping other factors and leading to increased anxiety-like behavior across several models.

Attempts to map the genetic factors involved in the regulation of anxiety-like behaviors have primarily utilized the quantitative trait loci (QTL) mapping

technique, in which the degree of association between genetic loci and quantitative measures are estimated (Belknap et al. 1997). Numerous studies that intercross selectively bred lines (Aguilar et al. 2002; Fernandez-Teruel et al. 2002; Flint et al. 1995; Turri et al. 2001a,b), inbred lines (Clement et al. 1997, Cohen et al. 2001, Gershenfeld & Paul 1998, Plomin et al. 1991, Yoshikawa et al. 2002), or outbred lines (Talbot et al. 1999) have implicated various loci in various anxiety-related tasks (for review, see Flint 2003). In mice, a locus on chromosome 1 has been the most consistently reported as being associated with anxiety-related behaviors (Flint et al. 1995, Gershenfeld & Paul 1998, Talbot et al. 1999, Turri et al. 2001a). A careful analysis of QTLs relevant to several different anxiety-related tasks, however, suggests that this locus may link more closely to variables related to exploration, rather than to anxiety per se (Turri et al. 2001a). For example, the chromosome 1 site was associated with both open-arm entries and closed-arm entries, whereas another site in that study (on chromosome 15) was more specifically associated with open-arm entries. QTLs have also been identified in studies of the rat, but these do not share homology with any of the identified regions in the mouse (Fernandez-Teruel et al. 2002). As of yet, linkage studies have not led to the identification of any specific genes involved in the regulation of anxiety-like behaviors.

The Role of Environment

Recent, compelling studies provide concrete evidence that the environment plays a crucial role in the establishment of anxiety-like behaviors. Francis et al. (2003) set out to determine whether and when maternal behaviors might influence the development of strain-specific differences in anxiety-like behavior. In their hands, BALB/cJ (BALB) mice behave as if they are more anxious than C57BL/6J (C57) mice, making fewer entries into and spending less time in the open arms of the elevated plus maze. To test the effects of prenatal and postnatal experience, C57 embryos were transferred into BALB or C57 dams. After birth, the pups were then cross fostered to BALB or C57 dams and tested in the elevated plus at three months of age. Only those C57 pups raised by BALB dams both in utero and postnatally took on the more anxious phenotype of their BALB foster mothers. Neither pre- nor postnatal maternal influences alone were sufficient to cause the C57 pups to develop BALB-like levels of anxiety-related behaviors (Figure 3).

A number of studies have demonstrated that maternal behavior has an important influence on adult anxiety-like behavior. Maternal separation for several hours a day during the early postnatal period results in adults that display increased anxiety-like behaviors as well as increased hormonal reactivity to stress (Huot et al. 2001, Kalinichev et al. 2002). Briefer separation actually leads to decreased anxiety-like behaviors and hormonal reactivity (Meaney et al. 1996, Vallee et al. 1997). This latter effect is primarily due to the maternal behavioral response to such separation. Dams respond to brief separation with higher levels of licking, grooming, and arched-back nursing (LG-ABN) (Liu et al. 1997). Indeed, pups
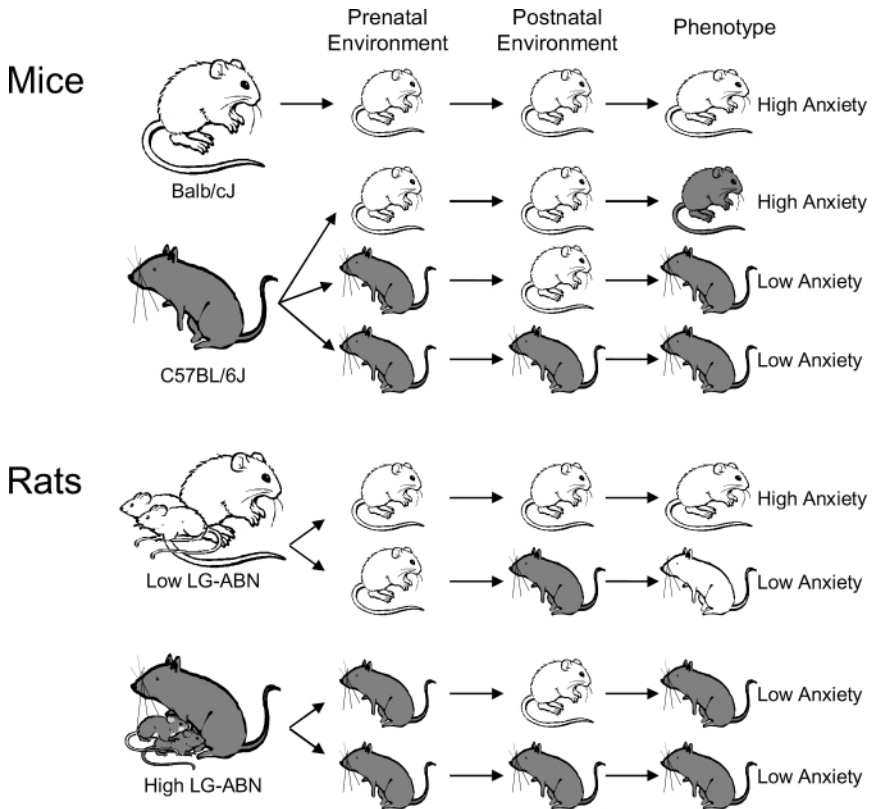
Figure 3 Epigenetic modes of inheritance. In mice and rats, anxiety-like behavior is influenced by pre- and postnatal maternal rearing. In mice (*upper pane*), both prenatal and postnatal cross fostering are required to convert a low anxiety C57BL/6J pup into a high-anxiety adult (Francis et al. 2003). In rats (*lower pane*), postnatal cross fostering is sufficient to convert a high-anxiety, low licking, grooming, and arched-back nursing (LG-ABN) pup into a low-anxiety, high LG-ABN adult. As in mice, postnatal cross-fostering alone does not convert a low-anxiety pup into a high-anxiety adult (Liu et al. 2000). *Shading* indicates genotype, *posture* indicates phenotype.

that have not been separated, but are simply raised by mothers that display high LG-ABN behaviors, have lower levels of anxiety as adults than pups raised by low LG-ABN mothers (Caldji et al. 1998). Cross fostering offspring of low LG-ABN mothers to high LG-ABN mothers causes the pups to develop low-anxiety-like behavior as adults (Liu et al. 2000). However, the converse is not true. Offspring of high LG-ABN mothers raised by low LG-ABN mothers do not show high-anxiety-like behavior, which suggests that prenatal experience or genetic endowment in the high LG-ABN offspring protect them from the effects of low LG-ABN mothering

(Figure 3). Of note, Francis et al. (1999) have shown that the effect of high licking-and-grooming can be inherited. Females cross-fostered to high LG-ABN mothers themselves become high LG-ABN mothers and go on to produce low-anxiety offspring regardless of whether their biological mother showed low or high LG-ABN behavior.

These studies on pre- and postnatal inheritance of maternal behavior and anxiety phenotype suggest that adult anxiety-like behavior is heavily influenced by events that occur during pre- and postnatal development. Interestingly, the data suggest that the dependency on pre- and postnatal experience is not symmetric. From the mouse data, making low-anxiety C57 pups more anxious requires both pre- and postnatal experience with a more anxious dam (Francis et al. 2003). This is in rough agreement with the rat data, where postnatal cross fostering does not convert low-anxiety pups into high-anxiety adults (Liu et al. 2000). High-anxiety rat pups, however, can be converted to low-anxiety adults through postnatal fostering alone (Liu et al. 2000). It would be interesting to know if the same is true in mice; the effects on anxiety of cross fostering high-anxiety BALB pups with low-anxiety C57 dams have not yet been reported. It is known, however, that postnatal cross fostering of BALB pups with C57 dams yields adults with C57-like learning abilities. The reverse, postnatal cross fostering of C57 pups with BALB dams, does not yield adults with BALB-like learning (Anisman et al. 1998). Thus the data so far are consistent with the hypothesis that aspects of the prenatal environment in C57 mice, and high LG-ABN rats, not only establish long-lasting behavioral patterns, but also protect against later exposure to BALB mice, or low LG-ABN rats. C57 mice display high LG-ABN behaviors, further tightening the analogy to the rat work (Anisman et al. 1998). This hypothesis leads to the prediction that prenatal embryo transfer of high LG-ABN rat pups into the uterus of low LG-ABN mothers would yield adults with increased anxiety-like behavior; and that postnatal cross fostering of BALB pups with C57 dams would yield low levels of anxiety-like behavior.

MOLECULAR GENETIC MANIPULATIONS

Maternal behavior tells only part of the story. Cross-fostering experiments with the HA- and LA-selected rat lines discussed above (Landgraf & Wigger 2002) clearly demonstrate a genetic mode of transmission, as pups from either line retain their biological parents' anxiety phenotype (Wigger et al. 2001). The QTL mapping data also strongly argue for genetic control of anxiety in both rats and mice. Finally, numerous genetic manipulations have direct and profound effects on anxiety-like behaviors (for a recent comprehensive review see Finn et al. 2003).

Indeed, the dizzying array of knockouts and transgenics that demonstrate abnormal levels of anxiety-like behaviors is rather daunting. Many fit neatly into preexisting theoretical constructs of anxiety. For example, mice deficient in the GABA-synthetic enzyme GAD-65 have reduced levels of the neurotransmitter

and show increased levels of anxiety-like behavior in the elevated zero maze and open field (Kash et al. 1999). GAD-65 knockouts are also less sensitive to the behavioral effects of benzodiazepines (Kash et al. 1999). These data are consistent with the known role of GABA in the anxiolytic effect of benzodiazepines and the hypothetical role (based on this pharmacological evidence) for endogenous GABA in the regulation of anxiety. Likewise, mice with alterations in various components of the GABAergic, serotonergic, noradrenergic, and adrenocortical systems have been found to have abnormal levels of anxiety-like behavior. Several reviewers have capably organized and discussed these and other knockouts in a comprehensive fashion (Clement et al. 2002, Finn et al. 2003, Flint 2003, Holmes 2001, Toth 2003).

Rather than confirming or extending existing hypotheses, data from other mutant mouse lines are more surprising. Anxiety-related phenotypes have been found in mice lacking such diverse genes as those encoding substance P receptor (Santarelli et al. 2001), preproenkephalin (Konig et al. 1996), neuropeptide Y (Inui et al. 1998), interferon (Kustova et al. 1998), dopamine D4 receptor (Dulawa et al. 1999), glucocorticoid receptor (Tronche et al. 1999), the α-isoform of calcium/calmodulin-dependent protein kinase II (αCaMKII) (Chen et al. 1994), protein kinase Cγ (Bowers et al. 2000), apolipoprotein E (Raber et al. 2000), neuronal cell-adhesion molecule (NCAM) (Stork et al. 2000, Stork et al. 1999), and dystrophin (Vaillend & Ungerer 1999). This group of genes is comprised of elements of virtually all the diverse functions of neurons and neuronal assemblies, from signaling molecules, through receptors and intracellular effectors, to cytoskeletal and extracellular matrix molecules (Wood & Toth 2001). The overall message one takes away from these studies is that normal anxiety requires normal neuronal functioning. Disrupt such functioning in any of a number of different ways and anxiety-like behavior is likely to be disrupted—not a very specific or informative conclusion.

Part of the specificity problem stems from the nature of knockout technology, and part from the limited degree to which most of these knockouts have been studied. Using the straightforward, first-generation knockout technology, genes are deleted wholesale from the mouse genome, eliminating expression throughout the life cycle and throughout the entire brain (not to mention the rest of the mouse). In most of these studies, therefore, it is unclear whether lack of the molecule during development or in the adult causes the phenotype. It is also unclear where in the brain (or elsewhere) expression of the gene product is required for normal anxiety-like behavior. It is important to know what neural systems the knockout affects because compensatory changes in any number of systems might result from the wholesale deletion of a gene during development or adulthood. Reviewed here are studies of four different lines of genetically modified mice, which, through use of innovative technology and/or careful study, have avoided or even taken advantage of the limitations of traditional knockout studies. The results from each enrich our understanding of anxiety and its treatment and illustrate the kinds of questions that genetic manipulation is perhaps best suited to answer.

The α-Subunit of the GABA$_A$ Receptor: Using Knock-in Technology to Demonstrate Isoform Specificity

Members of the benzodiazepine class of anxiolytic drugs derive their efficacy from binding and modulating the activity of the ionotropic GABA$_A$ receptor. In addition to reducing anxiety, these drugs also cause sedation, motor impairment, muscle relaxation, and amnesia, side effects that limit the use of these powerful anxiolytics (Buffett-Jerrott & Stewart 2002). Benzodiazepines bind the α-subunit of the pentameric receptor, enhancing the efficacy of GABA in activating the receptor (Mohler et al. 2001). Of the six isoforms of the α-subunit, two are insensitive to benzodiazepines; these two lack a conserved histidine residue found in each of the four benzodiazepine-sensitive isoforms (Mohler et al. 2002). Benzodiazepine-sensitive isoforms can be rendered insensitive by replacing this histidine with arginine (Wieland et al. 1992). In order to test the subunit specificity of the various behavioral effects of benzodiazepines, the histidine to arginine mutation was introduced by homologous recombination into the genes encoding each of the $\alpha 1$, $\alpha 2$, and $\alpha 3$ subunits (Low et al. 2000, Rudolph et al. 1999). In each case, the knock-in mutation rendered the mutated subunit unable to bind the benzodiazepine diazepam without affecting the expression of the mutated or other α isoforms.

Behavioral testing revealed that the $\alpha 2$ isoform is responsible for the anxiolytic effects of benzodiazepines in mice. In the elevated plus maze and light/dark choice tests, rendering the $\alpha 1$ or $\alpha 3$ isoform insensitive to diazepam via the histidine to arginine substitution did not prevent the drug from exerting its anxiety-reducing effect (Low et al. 2000, Rudolph et al. 1999). However, the same substitution in the $\alpha 2$ subunit eliminated the anxiolytic-like effect of diazepam in these tests (Low et al. 2000). Moreover, mice carrying the mutated $\alpha 2$ subunit were still sensitive to the sedating and motor-impairing effects of the drug (Low et al. 2000). Mice carrying the mutated $\alpha 1$ subunit were insensitive to the sedating and amnestic influences of the drug (Rudolph et al. 1999). These data demonstrate that the $\alpha 2$-containing GABA$_A$ receptor mediates the anxiolytic, but not the sedative or amnestic, effects of diazepam. These results have direct clinical relevance, in that they encourage the search for isoform-specific medications; a drug specific for the $\alpha 2$ subunit would be expected to reduce anxiety with little or no sedation (Mohler et al. 2002).

Importantly, the results also have implications for the basic neurobiology of anxiety, as the distribution of the receptor indicates where in the brain benzodiazepines might exert their anxiolytic effect. The $\alpha 2$ isoform is expressed mostly in the limbic system and cortex (Fritschy & Mohler 1995, Pirker et al. 2000). The subregional and subcellular localization of the isoform may be of even greater significance. Within the amygdala, the $\alpha 2$ isoform is expressed primarily in the central nucleus (Kaufmann et al. 2003). Analysis of subregional expression in other areas would help refine hypotheses regarding the circuits capable of modulating anxiety. Within pyramidal neurons, the $\alpha 2$ isoform is preferentially localized within the axon initial segment, where it can play a crucial role in the output from these

neurons (Fritschy & Mohler 1995, Nusser et al. 1996). A simple hypothesis consistent with these data is that output from the central nucleus promotes anxiety-like behavior; benzodiazepines, by enhancing inhibition onto the axonal initial segment, reduce that output. As tools develop for manipulating genes with increasing tissue specificity, one could imagine testing this hypothesis, perhaps by directing expression of a wildtype $\alpha 2$ isoform to the amygdala of the knock-in mouse carrying the $\alpha 2$ arginine substitution. Demonstrating anxiolytic efficacy in such a mouse would directly prove a role for the amygdala in the action of benzodiazepines.

Protein Kinase Cε: Studying the Downstream Effects of a Knockout Reveals Influences on Other Systems

Combining multiple neurobiological approaches to the study of a knockout of the ε isoform of protein kinase C (PKCε) has helped identify a novel mechanism in the regulation of anxiety. PKCε is a serine/threonine kinase expressed widely in the nervous system and other tissues (Saito et al. 1993). Because of data suggesting a possible role of PKCε in mediating the effects of alcohol, the sensitivity of PKCε knockout mice to alcohol was measured and found to be increased (Hodge et al. 1999). Further study revealed that these mice were supersensitive to various allosteric modulators of the GABA$_A$ receptor, including benzodiazepines, barbiturates, and neurosteroids (Hodge et al. 1999, 2002). These data suggest that PKCε acts to desensitize the GABA$_A$ receptor to its modulators. To determine if this putative effect of the kinase was dependent on adult or developmental activity, the authors used a peptide that inhibits translocation of the kinase to the periphery, blocking its activity (Yedovitzky et al. 1997). Inhibiting PKCε with this peptide significantly increased the benzodiazepine-induced enhancement of GABA$_A$ function in cortical microsacs derived from wildtype, but not knockout, mice, arguing strongly that the kinase acts in the adult to diminish sensitivity to GABA$_A$ receptor modulators.

Intriguingly, when the PKCε knockouts were placed in the elevated plus maze and open field, they made more entries to the open arms and center of the open field, respectively, as if they were less anxious (Hodge et al. 2002). Low doses of bicuculline normalized the behavior of the knockouts in these tests but had no effect on wildtype control animals (Hodge et al. 2002). The anxiety phenotype of these mice, therefore, is likely related to the enhancing effect of the gene deletion on GABA$_A$ receptor function. The simplest interpretation of these data is that the knockout enhances GABA$_A$ receptor sensitivity to an endogenous modulator (Gordon 2002, Hodge et al. 2002). The most likely candidates for such an endogenous modulator are the neurosteroids (Baulieu et al. 2001, Gordon 2002). Those neurosteroids that enhance GABA$_A$ receptor function are also anxiolytic in the elevated plus maze (Melchior & Ritzmann 1994); the anxiety-related effects of neurosteroids can be prevented by the GABA$_A$ antagonist, picrotoxin (Baulieu et al. 2001). Abnormal levels of neurosteroids have even been detected in patients suffering from anxiety and depression (Bicikova et al. 2000, Spivak et al. 2000). The combination of

behavioral, pharmacological, and neurophysiological approaches to the study of this genetically manipulated organism allowed the authors to go beyond the simple statement that the knockout was less anxious. This combination of approaches led to the recognition that PKCε plays a role in regulating the sensitivity to endogenous modulators of anxiety and suggests the kinase as a target for anxiolytic therapy.

CRH Receptor 1: Using Tissue Specificity to Localize Function

Stress and anxiety have long been thought of as tightly linked, although numerous exceptions can be found to this general rule (File 1996). In many cases, anxiety-provoking situations increase, and benzodiazepines decrease, the activation of the hypothalamus-pituitary-adrenal (HPA) axis, the primary mediator of stress hormone release (Le Fur et al. 1979, Pellow et al. 1985). Corticotrophin-releasing hormone (CRH) is the primary regulator of HPA activity. Stress induces the release of CRH from the hypothalamus, which causes the release of adrenocorticotropin (ACTH) from the pituitary into the general circulation. ACTH then induces the release of glucocorticoid stress hormones from the adrenal glands (Miller & O'Callaghan 2002). CRH also modulates anxiety when injected directly into the brain (Dunn & Swiergiel 1999, Eckart et al. 1999, Martins et al. 1997). CRH activity is mediated by at least two receptors, CRH receptors 1 and 2 (CRH-R1 and CRH-R2) (Eckart et al. 2002). Both receptors also bind urocortin, a peptide related to CRH (Vaughan et al. 1995). CRH, urocortin, and its receptors are expressed in numerous parts of the central nervous system, and there is evidence to suggest they have roles in processes other than the stress response (Chang & Opp 2001, Drolet & Rivest 2001, Eckart et al. 1999, Vaughan et al. 1995, Wood & Toth 2001).

The relationship between CRH and anxiety has been tested in several different lines of genetically modified mice (Finn et al. 2003). Mice lacking CRH behave similarly to wildtype controls in the elevated plus maze and open field (Dunn & Swiergiel 1999). Mice overexpressing CRH, however, have increased anxiety-like behaviors in the elevated plus maze and light/dark test (Heinrichs et al. 1997, Stenzel-Poore et al. 1994, van Gaalen et al. 2002). Two different lines of mice lacking CRH-R1 have been produced, and both have decreased anxiety-like behavior in the elevated plus and light/dark tests (Smith et al. 1998, Timpl et al. 1998). Data on anxiety measures in CRH-R2 knockout mice are inconsistent across three different laboratories and several different tests (Bale et al. 2000, 2002; Coste et al. 2000; Finn et al. 2003; Kishimoto et al. 2000). The effect on anxiety of deleting the gene for urocortin has also been inconsistent. One group reported an anxiogenic effect in the elevated plus maze and open field, but not the light/dark test (Vetter et al. 2002), whereas a second group reported no effect on these three tests (Wang et al. 2002). Although the reasons are unclear why different groups report different results, the data do suggest that CRH or a related ligand acts on CRH-R1 to increase anxiety-related behaviors.

These studies left open the long-standing question of the localization of the role for the CRH system in anxiety: Are the CRH effects on anxiety due to activation

of the HPA axis or to CRH neurotransmission in the forebrain? To address this question, Müller et al. (2003) used a tissue-specific knockout strategy to delete the CRH-R1 gene from the forebrain, including limbic areas, but not from the HPA axis. Using the *cre*/*loxP* system, the investigators used the αCaMKII promoter to drive expression of the Cre recombinase to delete exons 9–13 of the CRH-R1 gene from the forebrain, sparing the hypothalamus and pituitary (although the authors did not specifically show high-magnification views of the HPA axis in the paper). Unlike the original CRH-R1 knockout, which has a dramatically reduced HPA axis response to stress, the tissue-specific knockout has slightly increased ACTH and corticosterone responses to stress (Müller et al. 2003, Timpl et al. 1998). Nonetheless, the tissue-specific knockout has the same anxiety phenotype as the complete knockout, with substantial increases in entries into the open arms of the elevated plus maze and decreased latency to enter the dark area in the light/dark test (Müller et al. 2003). These data differentiate the role of the CRH system in regulating the HPA axis response to stress, which involves primarily pituitary CRH-R1 and its role in regulating anxiety-like behavior, involving primarily forebrain CRH-R1. Confirmation of this dichotomy with a tissue-specific knockout of pituitary CRH-R1 would be a logical and satisfying next step.

5-HT1A Receptor: Temporal Specificity Defines a Developmental Critical Period

Of the several knockouts of individual serotonin receptors, the 5-HT1A receptor knockout has been the most informative with respect to anxiety. Three independent laboratories have produced 5-HT1A receptor knockout mice independently, each on a different background strain (Heisler et al. 1998, Parks et al. 1998, Ramboz et al. 1998). In each case, the knockout mice behave as if they are more anxious in the elevated plus (or zero) maze and the open field test. These data are consistent with the generally anxiolytic effects of 5-HT1A agonists (Griebel 1995). Antagonists of the 5-HT1A receptor, however, are not generally anxiogenic (Canto-de-Souza et al. 2002), which raises the question of whether the knockout effect is indeed due simply to the absence of the adult receptor rather than to some developmental or compensatory effect.

In order to clarify the dependence of the anxiety phenotype on the presence of the receptor in the adult, and to begin to define in which tissues expression of the receptor is required, Gross (2002) and colleagues developed a tissue-specific, inducible rescue of the 5-HT1A knockout. They first directed the insertion of a cassette containing a promoter responsive to the tetracycline-regulated transcriptional activator protein (tTA) into the 5′ leader sequence of the 5-HT1A receptor gene. This insertion ablated expression of the receptor by its native promoter. The line carrying this insertion was then crossed with a line carrying a transgene with the tTA protein expressed under the control of the αCaMKII promoter. The tTA protein successfully induced expression of the 5-HT1A receptor in postsynaptic target tissues, such as the hippocampus and cortex, but not in the serotonergic neurons of the

dorsal raphe nucleus. Rescue in this manner restored normal anxiety-like behavior, demonstrating that the lack of postsynaptic receptors, rather than presynaptic, causes the anxiety phenotype in the original knockout line.

To directly test when expression of the postsynaptic 5-HT1A receptor is required, Gross (2002) treated animals at various ages with tetracycline, which prevents activation via the tTA protein. When tetracycline was given only during adulthood, allowing 5-HT1A receptor expression only during development, the mice behaved like wildtype animals. When tetracycline was given throughout gestation and weaning, adult expression of the receptor was not able to rescue the anxiety phenotype, and the mice behaved like knockout animals (Figure 4). These surprising findings demonstrate that stimulation of postsynaptic 5-HT1A receptors during a developmental critical period is required to establish normal patterns of anxiety-like behavior that then persist into adulthood. They help clarify why 5-HT1A antagonists might not cause anxiety when given to adult animals. Moreover, they leave open the question of the relevance of adult 5-HT1A expression to anxiety.

Then why are 5-HT1A agonists anxiolytic in the adult? Intriguing new data combining further analysis of the 5-HT1A knockouts with more old-fashioned

Figure 4 Developmental rescue of a knockout anxiety phenotype. Timeline of rescue of 5-HT1A receptor expression (*upper panel*). In the elevated plus maze, developmental rescue (*lower left*) but not adult rescue (*lower right*) restores wildtype levels of anxiety-related behavior (Gross et al. 2002).

manipulations bears directly on this question (Santarelli et al. 2003). SSRIs induce neurogenesis, an effect that has been hypothesized to rely on activation of the 1A receptor (Gould 1999, Radley & Jacobs 2002). In wildtype mice, chronic, but not acute, fluoxetine or 8-OH-DPAT increases neurogenesis in the dentate gyrus and exerts anxiolytic-like effects in the novelty suppressed feeding paradigm (Bodnoff et al. 1988, 1989; Malberg et al. 2000; Santarelli et al. 2003). The neurogenic and behavioral effects of both agents are blocked in the 5-HT1A receptor knockouts (Figure 3) (Santarelli et al. 2003). Santarelli et al. (2003) also used hippocampal-specific x-irradiation to ablate neurogenesis without otherwise altering serotonergic neurotransmission. They found that ablation of hippocampal neurogenesis prevented the behavioral effects of fluoxetine. These data suggest that chronic SSRI treatments exert their anxiolytic effect through the activation of 5-HT1A receptors, which in turn increase neurogenesis in the dentate gyrus of the hippocampus. It is unclear as of yet whether the plasticity-inducing effects of stimulating 5-HT1A receptors in the adult are related to those of stimulating the receptors during development.

Lessons from Genetically Modified Mice: Power Depends on Specificity and Depth

These selected studies demonstrate the power of using genetically modified mice to probe the neurobiological mechanisms underlying anxiety. Increasing tissue and temporal specificity allows one to test specific hypotheses—the relevance of the HPA axis to the effects of CRH on anxiety, or the isoform-dependence of the benzodiazepine effect—that would be difficult if not impossible to study with traditional pharmacological or neurobiological tools. At the same time, applying these traditional tools to the study of genetically modified animals allows one to begin understanding why a given genetic lesion causes a given behavioral phenotype. The explanatory power of a given genetic model, therefore, depends on the specificity of that model, the depth of knowledge of any secondary effects, and, of course, the specificity of the question being asked.

FROM MOUSE, BACK TO HUMAN

One advantage of the genetic approach to anxiety is that animal model data can be readily used to probe human anxiety disorders. One recent attempt tested the hypothesis that QTLs regulating anxiety-related behavior in the mouse would also be associated with anxiety disorders in humans (Smoller et al. 2001a). Mouse QTLs from a variety of studies of anxiety models were used to determine the homologous regions on human chromosomes. Polymorphisms from these regions were then genotyped in members of a large, multigenerational pedigree with a high incidence of panic disorder. Two regions of possible association were found: one on chromosome 12, corresponding to the region of mouse chromosome 15, linked to open-arm entries in the elevated plus maze (Turri et al. 2001a); and a weaker,

broader region on chromosome 1, corresponding to the same chromosome in the mouse, the region most consistently reported in mouse studies (Flint et al. 1995; Gershenfeld & Paul 1998; Talbot et al. 1999; Turri et al. 2001a,b). This same group has utilized a candidate gene approach in a similar fashion. Five candidate genes were chosen from the broad array of knockouts that result in abnormal anxiety and were used to search for linkage to the anxiety-related trait of behavioral inhibition in children (Smoller et al. 2001a, Smoller et al. 2004). Associations were found between behavioral inhibition and the genes for GAD-65 and CRH. While these initial studies await confirmation, they demonstrate the potential power of genetics to cross species barriers.

Of course the principle goal of bridging animal and human studies involves the development of new treatments. Some of the studies mentioned above have the potential to result in new treatments for anxiety. Agents specific to α2-subunits of the GABA$_A$ receptor are already under study, in an effort to improve upon benzodiazepines (Atack 2003; Collins et al. 2002; Griebel et al. 2001, 2003). CRH antagonists are under study as well, as are specific CRH-R1 antagonists (Li et al. 2003, Oshima et al. 2003, Seymour et al. 2003). Looking into the future, specific PKCε inhibitors might treat anxiety by making GABA$_A$ receptors more sensitive to endogenous anxiolytics, and novel agents, which promote hippocampal neurogenesis, might be used to treat anxiety. Further into the future, one can imagine developing a sophisticated understanding of the circuit- and systems-level effects of the absence of the 5-HT1A receptor during development and harnessing that understanding by developing agents that could counteract those effects. These possibilities are a sampling of the potential contributions of genetic approaches to the study of anxiety.

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