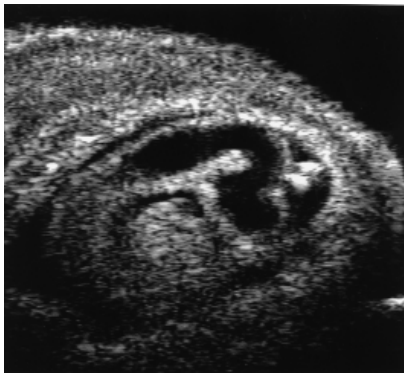

Fig. 2. Sagittal ultrasound image of an E9.5 mouse embryo about to be injected with a high-titer retroviral stock. The ventricular system of the brain appears as a curved black space in the center of the image.

brain nuclei, all begin long before most neurons and glia are generated (see ref. 6 for review). We now have an enormous list of genes that are expressed in patterns suggesting involvement in these processes. These data, plus the morphological development of the embryonic forebrain, suggest that much of the patterning occurs between roughly E9 and E13. This new method may therefore be enormously valuable for manipulating the brain at a critical time during its development.

Although this is primarily a techniques paper, it also provides some interesting insights into the phenomenon of 'retroviral silencing'[7]. If undifferentiated embryonic carcinoma (EC) cells are infected with retrovirus, the provirus integrates into the host cell DNA as expected, but its expression is somehow blocked. However, if the cells are made to differentiate before infection, the virus is expressed. Somehow, the virus must be 'silenced' when it infects an undifferentiated cell, such that it cannot be reactivated even by differentiating the cell into a permissive state. There are a few exceptions, but most can be attributed to viral mutations or the effects derived from the site of insertion of the retrovirus[7]. How silencing works is not entirely understood, but it seems to be a two-stage process involving the viral long terminal repeat (LTR), the part of the viral genome that contains the endogenous retroviral promoter[8]. First the retroviral enhancer (the part of the promoter that drives tissue-specific expression) is turned off, probably because a 'stem cell factor' binds to the LTR[9]. Subsequently, the site is methylated, this being a well-known mechanism of inactivating genetic loci. Whatever the molecular details, once the construct is turned off, it stays off even if the cell subsequently differentiates.

We have long suspected that neural precursor cells, like EC cells, silence retroviral promoters. For instance, E9 mouse embryos fail to express retrovirally transduced genes in the neural plate, even though the same construct can be expressed in mesodermal tissues[10]. The answer, as Gaiano *et al.* demonstrate clearly, is to use a vector in which expression is not driven by the LTR, but by a separate internal promoter.

If retroviral silencing as a practical problem can be overcome so easily, then why is it so interesting? Silencing probably tells us something about how tissue-specific enhancers are controlled during the process of differentiation. During neurogenesis, the precursor cells from which neurons are derived are found in the ventricular zone (VZ), the germinal layer of the forebrain. The VZ cells (unlike postmitotic neurons) are infected by an injection of virus into the cerebral vesicle, because they are mitotic and because virus penetrates poorly into tissue, so only the VZ cells will be exposed to the virus. Yet when Gaiano *et al.* infected at E9.5 and analyzed five days later with a virus driven from the LTR, they found expression in postmitotic neurons but not in VZ cells. Presumably the virus remains silenced in the undifferentiated precursor cells of the VZ but is activated in neurons derived from the infected VZ cells. How do differentiating neurons escape the block? Perhaps VZ cells express the stem cell factor but do not perform the methylation step. Perhaps, methylation occurs but can be reversed in neurons. Either way, it would be surprising if this unknown control mechanism was not telling us something important about the control of differentiation during neurogenesis. It would be very interesting to know whether VZ cells express a stem cell factor similar to that expressed by EC cells.

In the meantime, though, neuroembryologists will want to evaluate this novel approach to gain-of-function studies. The most important question is whether the technique is cheap and easy enough to encourage its widespread adoption. For that we will have to wait and see.

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Anxiety: at the intersection of genes and experience

Stephan G. Anagnostaras, Michelle G. Craske and Michael S. Fanselow

Human anxiety disorders arise from a combination of genetic vulnerability and traumatic experience. Mice with a $GABA_A$ receptor mutation may provide a model for these disorders.

Fear is an intense emotional experience because of its critical function in organizing responses necessary for survival[1]. Normal anxiety is evolutionarily adaptive and experienced by everyone at times, but excessive fear is debilitating and may indicate an anxiety disorder. In this issue of *Nature Neuroscience*[2], Florence Crestani and her coworkers report that mice heterozygous for deletion of the gene encoding the γ2 subunit of the $GABA_A$ receptor show substantially enhanced anxiety in many situations. This profile led the authors to suggest that these mice may provide an animal model of anxiety disorders.

An estimated 25% of the population are afflicted with an anxiety disorder at some time in their lives. These disorders, which include specific phobias as well as panic disorder and post-traumatic stress disorder

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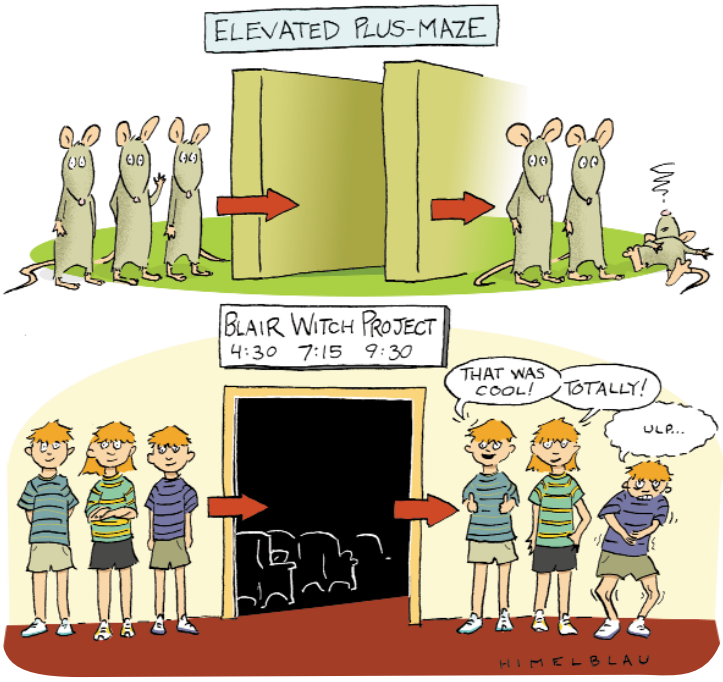

Fig. 1. Individual variability in response to anxiety-provoking environments. Top, several mice are placed in an elevated plus maze, in which two of the four arms have no walls. Most mice tentatively explore the open arms (albeit to a lesser degree than the closed arms). However, one mouse experiences more severe anxiety in the apparatus and completely avoids the open arms. In Crestani *et al.*'s experiment, the anxious mouse would be heterozygous for the γ2 mutation, and the others would be wild type. Bottom, a group of friends go to the movies to see the latest horror film. All but one enjoy the movie. That individual gets extremely frightened, now jumps at the slightest noise and has persistent nightmares.

(PTSD), are distinct from the fears that many people experience in response to (say) spiders, heights or public speaking. In contrast to these common fears, anxiety disorders lead to significant impairment to normal life, often accompanied by great personal distress and societal costs. One person suffering from anxiety described her symptoms as follows:

"*I am new here, and I wanted to mention the physical symptoms that I get: tingling, sharp pains, light-headedness, chest discomfort, indigestion, lump in my throat, feelings that something is going to happen to me right now, feelings of unreality, skin crawling, heart pounding, smothering feeling when I drive—I'm not sure if I'm going to make it, can't watch the news or read the papers, because I get too depressed about anyone being sick or dead, and the worst for me, the heart palpitations.*"

What causes anxiety disorders? Many phobias originate with specific traumatic events, experienced either directly or vicariously, such as being attacked by a dog or seeing someone else being attacked or being afraid of dogs. However, traumatic events do not inevitably cause persistent anxiety; for example, only 35% of women who are raped go on to develop PTSD[3]. Although some of this variability is due to differences in life history, some is likely to be genetic in origin. Among combat veterans, for instance, the concordance ratio for PTSD is higher among monozygotic than dizygotic twins[4], even after controlling for degree of combat exposure[5]. A similar pattern of concordance holds for most anxiety disorders, including susceptibility to phobias. However, even given a genetic predisposition, subjects necessarily must experience a trauma to develop these disorders (**Fig. 1**). The anxiety disorders seem to share a common genetic risk factor, whereas the particular fear that emerges (for example, social situations, confined spaces or animals) is largely attributable to individual life experiences[6,7]. This nonspecific genetic vulnerability has been interpreted as a lowered threshold for limbic system activation or excessive readiness to perceive stimuli as threatening that manifests as elevated autonomic arousal and harm avoidance[8].

One candidate for this genetic risk factor is a defective response to GABA, the primary inhibitory neurotransmitter in the brain. Disorders of inappropriate or excessive anxiety can be treated with benzodiazepine agonists, such as Valium, which bind to the α subunit of the $GABA_A$ receptor and increase inhibition. There is considerable evidence for abnormal GABA receptor function in panic disorder[9]. Patients with anxiety disorders show reduced sensitivity to benzodiazepine agonists and increased sensitivity to antagonists. Brain imaging studies with radiolabeled ligands found decreased benzodiazepine receptor binding in several cortical areas of panic patients. Moreover, two areas that have been implicated in rodent fear conditioning, the amygdala and hippocampus, have particularly high concentrations of $GABA_A$ receptors.

These findings led Crestani and colleagues[2] to suspect that the altered GABAergic function[10] in mice heterozygous for deletion of the γ2 subunit may make these mice an appropriate model of human anxiety disorders. Unlike previous rodent models produced by selective breeding for anxiety-related behaviors, the γ2 knockout mice have a clearly defined mutation, targeted to a mechanism implicated in human panic disorder. Crestani *et al.*[2] demonstrated that these mice show enhanced anxiety in several established tests of innate and learned fear[1]. One test of innate fear is the elevated plus maze, in which an animal is placed at the cross between two elevated sets of arms, one closed (walled) and one open. Anxious mice tend to avoid the anxiety-provoking open arms, and the authors found that γ2-deficient mice spent less time in these open arms than wild-type mice. Benzodiazapine agonists normally reduce the signs of anxiety even in wild-type mice. However, Crestani *et al.*[2] used a low dose, which did not affect wild-type mice, and found that on three indices of unlearned fear, the drug eliminated the enhanced anxiety found in mutants, bringing them back to wild-type levels. This sensitivity to the anti-anxiety effects of benzodiazepine drugs is reminiscent of that found in human anxiety disorders, suggesting that the underlying mechanisms may be similar.

In addition to innate fear, the authors examined several variants of Pavlovian fear conditioning, in which behavioral trauma induces learned fear. Both the behavioral contingencies and the neural circuitry involved in this process have been extensively analyzed[1]. In standard fear conditioning, in which a brief tone is presented simultaneously with an aversive foot shock in a novel context, the γ2 mutants showed a normal conditional fear response to both the tone and the training context[2]. Howev-

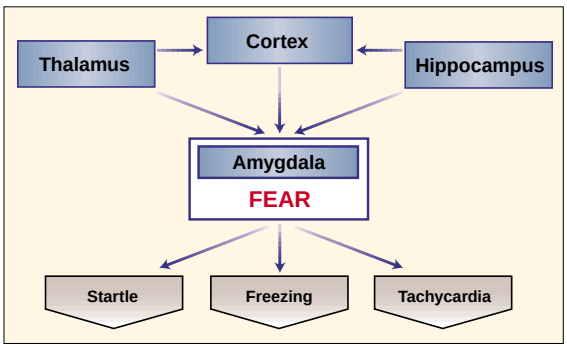

Fig. 2. Simplified neural circuit mediating fear. The amygdala receives multiple inputs and organizes a coherent defensive strategy to threat, including the example responses shown.

er, they showed enhanced fear in two other forms of fear conditioning. First, in trace conditioning, in which a one-second delay was inserted between the termination of the tone and onset of the shock, mutant mice showed enhanced conditioning relative to normal controls. Second, the authors tested the effect of decreasing the signal value of a cue, making it an imperfect predictor of foot shock. Normal mice show a reduced freezing response to this partially conditioned cue. In contrast, the mutant mice showed equally high levels of fear to the partially and normally conditioned cues, which can be regarded as inappropriate anxiety. Thus, these mice were predisposed to be hyperanxious but exhibited only selective alterations in learned fear[2]. These findings are in many ways reminiscent of the nonselective genetic predisposition proposed to exist for human anxiety disorders.

How does this γ2 mutation affect the circuitry involved in fear learning? Rats and humans with extensive damage to the amygdala seem to lose the capacity to experience fear, and the hippocampus is also involved in fear learning, probably via its descending projections to the amygdala[11-13] (**Fig. 2**). In generating anxiety, the amygdala requires converging information from several other structures, notably the hippocampus and cortex. Crestani *et al.*[2] found that the mutants showed pronounced reductions in benzodiazepine binding in cortical areas, the hippocampus and, to a lesser degree, the amygdala. Although benzodiazepines do not bind the γ subunit directly, GABA receptors lacking the γ subunit fail to cluster and may not localize properly to synapses, which may account for these results. In addition, two tasks that have been proposed to depend on the hippocampus, passive avoidance (see **Fig. 5c** of Crestani *et al.*[2]) and trace fear conditioning, were enhanced in the mutants. Based on these data, the authors suggest that the hippocampus (rather than the amygdala) is the probable source of increased anxiety in these animals.

We believe this conclusion to be premature for several reasons. Two well-established forms of hippocampus-dependent aversive learning, the Morris water maze and contextual fear conditioning[14], were normal in γ2 mutant mice. In addition, there is no consensus that passive avoidance behavior depends on the hippocampus (although some studies suggest it does), nor is it clear that trace fear conditioning at the short delay interval used in the present study requires the hippocampus. Thus, we believe that the preponderance of the behavioral evidence suggests that hippocampal circuits normally involved in learning are largely intact in these mutants, but that the amygdala—or one of its inputs—is overactive. Given that the mutation altered GABAergic function in hippocampus and cortex, it is possible that anxiety responses were altered because the amygdala received aberrant input from these regions. Obviously, additional work will be required to test this speculation.

Regardless of the circuitry involved, however, γ2 mutant mice may represent a good genetic model of some forms of anxiety predisposition because they are likely to overreact to many anxiety-provoking situations. Such genetic models[2,15] are important in furthering the study of innate contributors to anxiety disorders. First, the mice offer the promise of a genetic model of the anxiety-predisposed human, which may be useful in improving drug discovery. Rather than examining the effects of novel anxiolytics on normal rats, one may examine genetic models in hopes of finding agents selective for abnormal anxiety. Second, these mice offer easily testable predictions about mutations that may be found in anxiety patients. Finally, although the identification of genetic predisposing factors would certainly be a major advance, it is clear that genes alone will not explain human anxiety. These mutant mice should therefore be a valuable model for testing ideas about how genes and environment interact to produce this condition.

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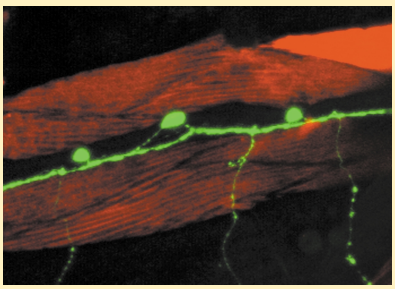
Synaptic physiology in *C. elegans*

Although *C. elegans* is a powerful genetic model, its one-millimeter length has made functional data difficult to obtain. In this issue (page 791), Richmond and Jorgensen report patch-clamp electrophysiology in *C. elegans* muscle. They combined physiology with genetic analysis to identify three receptors that function at the neuromuscular junction, a simple polyinnervated synapse. In this dissected worm, muscles are immunostained red, and GABAergic neurons of the ventral nerve cord express green fluorescent protein (photo by Jean-Louis Bessereau).



Sandra Aamodt