THE DEVELOPMENTAL ORIGINS OF ANXIETY

Cornelius Gross and Rene Hen‡*

Anxiety is a mental state that is elicited in anticipation of threat or potential threat. Sensations of anxiety are a normal part of human experience, but excessive or inappropriate anxiety can become an illness. In this review, we consider the evidence for anxiety as a product of early environmental experiences, the impacts of which are modulated by genetic susceptibility factors. We propose that such interactions can induce persistent structural and functional changes in the brain that underlie susceptibility to anxiety. Investigation of the molecular nature of these factors and the plastic changes that they induce has the potential to reveal why different individuals experience different levels of anxiety.

OBSESSIVE–COMPULSIVE DISORDER
A psychological disorder in which the person is burdened by recurrent, persistent thoughts or ideas, and/or feels compelled to carry out a repetitive, ritualized behaviour. Anxiety is increased by attempts to resist the compulsion and is relieved by giving way to it.

**Mouse Biology Programme, European Molecular Biology Laboratory, Via Ramarini 32, 00016 Monterotondo (Rome), Italy.*
‡Center for Neurobiology and Behavior, Columbia University, 722 West 168th Street, New York, New York 10032, USA.
e-mails: cornelius.gross@embl-monterotondo.it; rh95@columbia.edu
doi:10.1038/nrn1429

Anxiety is accompanied by a characteristic set of behavioural and physiological responses including avoidance, vigilance and arousal, which evolved to protect the individual from danger. These anxiety-related responses have been described in higher animals, and seem to be part of a universal mechanism by which organisms adapt to adverse conditions.

A growing body of data indicates that human susceptibility to mood disorders such as depression and anxiety can be determined early in life. These data support the view that early developmental mechanisms can set the lifelong tendency of an organism to express anxiety in response to threatening stimuli. Such developmental mechanisms are under both genetic and environmental control. Studies of anxiety-related behaviour in monkeys and rodents support the important role of gene–environment interactions in the aetiology of anxiety.

In its non-pathological form, anxiety can be divided into two categories: state anxiety, a measure of the immediate, or acute, level of anxiety; and trait anxiety, which reflects the long-term tendency of an individual to show an increased anxiety response. In its pathological form, anxiety can severely interfere with normal life, and has been classified into six disorders described in the *Diagnostic and Statistical Manual of the American Psychiatric Association*[1]: generalized anxiety disorder, social phobia, simple phobia, panic disorder, post-traumatic stress disorder (PTSD) and OBSESSIVE–COMPULSIVE DISORDER (OCD). Together, these disorders affect over 20% of the population at some point in their lifetime, with an annual estimated cost of $44 billion in the United States alone[2]. Despite the wide range of anxieties encompassed by these six disorders, all probably share common behavioural and physiological characteristics. This hypothesis stems mainly from the fact that most anxiety disorders respond to a similar spectrum of pharmacological treatments (TABLE 1).

In this review, we discuss the evidence supporting the idea that increased susceptibility to the expression of anxiety-related behaviour in humans, primates and rodents is the result of abnormal development. Our review focuses on recent studies that highlight the important interplay between genetic and early environmental factors in modulating anxiety-related behaviour.

Physiology of anxiety

Excessive anxiety has been treated primarily with drugs that have calming properties, including alcohol, barbiturates, opiates, beta-blockers and benzodiazepines[3]. Of these, the benzodiazepines are the most specific and effective, and are therefore widely used to treat both normal and pathological anxiety. Benzodiazepines increase the potency of the main inhibitory neurotransmitter in the brain, GABA (γ-aminobutyric acid), by modulating the function of $GABA_A$ receptors[4]. On the basis of the

Table 1 | Categories of anxiety disorders, their prevalence and most common treatments*

Disorder	Symptoms	Lifetime prevalence (%)	Treatments
Generalized anxiety	Unrealistic, excessive and long-lasting worry, motor tension, restlessness, irritability, difficulty sleeping, hypervigilance	5	Benzodiazepines, SSRIs, venlafaxine, buspirone, cognitive/behavioural therapy
Panic disorder (often associated with agoraphobia)	Brief, recurrent, unexpected episodes of terror (peak within 10 min), sympathetic crises, dyspnoea (shortness of breath), fear of dying and losing control, de-realization	3	SSRIs, benzodiazepines, cognitive/behavioural therapy
Post-traumatic stress disorder	Following an extremely stressful event (involving actual or threatened injury), recurrent episodes of fear often triggered by reminders of initial trauma (re-experiencing and avoidance), autonomic arousal	3	SSRIs, cognitive/behavioural therapy
Social phobia	Aversion, fear, autonomic arousal in unfamiliar social settings	13	SSRIs, benzodiazepines, cognitive/behavioural therapy
Specific phobia	Aversion, fear, autonomic arousal in specific situations (exposure to animals, blood and so on)	11	Behavioural therapy (exposure)
Obsessive–compulsive disorder	Recurrent obsessions and compulsions: obsessions are persistent, intrusive or inappropriate thoughts that cause anxiety; compulsions are repetitive acts that the sufferer feels driven to perform to alleviate anxiety	2	SSRIs, behavioural therapy

*As described in DSM-IV (REF. 1) and in REF. 76. DSM-IV, *Diagnostic and Statistical Manual of the American Psychiatric Association* Vol 4; SSRI, selective serotonin re-uptake inhibitor.

effectiveness of GABA-enhancing drugs, it has been proposed that excessive excitatory neurotransmission is an important physiological hallmark of anxiety[5]. However, the precise anatomical location and nature of this brain hyperexcitability are not known. Functional magnetic resonance imaging (fMRI) studies of humans with anxiety disorders have revealed increased baseline activity in the cingulate cortex and parahippocampal gyrus[6], and increased brain activity in response to anxiety-provoking stimuli in the AMYGDALA, parahippocampal gyrus and frontal cortex (reviewed in REF. 7). Consistent with imaging data, surgical removal of portions of the cingulate cortex is an effective treatment for refractory OCD[8]. Together, these studies indicate that the forebrain might be a site of increased excitatory neurotransmission in anxiety disorders. Studies of mice with genetically engineered $GABA_A$ receptors that specifically lack the benzodiazepine-binding site showed that $GABA_A$ receptors that contain the $\alpha 2$ subunit and that are located in the hippocampus, cortex and amygdala are primarily responsible for the anxiolytic effects of these drugs[9] (see REF. 10 for a review of animal models of anxiety).

In the past two decades, another class of drugs, the selective serotonin re-uptake inhibitors (SSRIs), have replaced the benzodiazepines as first-line treatment for anxiety, mostly because they lack the addictive properties of benzodiazepines[11]. SSRIs, such as fluoxetine hydrochloride (Prozac; Eli Lilly), sertraline (Zoloft; Pfizer), citalopram (Celexa; Forest Pharmaceuticals) and paroxetine hydrochloride (Paxil; GlaxoSmithKline), are now used effectively to treat most anxiety disorders. They probably act by selectively blocking the re-uptake of serotonin (5-HT) following its release from neurons, thereby increasing the potency of 5-HT neurotransmission in the brain[12]. Although the physiological consequences of this increased potency are still not well understood, functional imaging studies show that SSRI treatment can dampen brain excitability[13].

An important difference between the modes of action of benzodiazepines and SSRIs is their kinetics in the brain. Benzodiazepines act rapidly, within minutes of administration, whereas SSRIs act much more slowly. The therapeutic effects of SSRIs only become apparent between two and four weeks after the commencement of treatment. This slow therapeutic onset implies that the anxiolytic effect of SSRIs depends on inducing gradual changes in brain structure or function[14]. In serotonergic neurons, slow desensitization of auto-receptors contributes to a gradual increase in 5-HT neurotransmission following SSRI treatment[15]. In the forebrain, the expression profile of several molecular markers gradually changes during SSRI treatment. Recently, proliferation of new neurons in the rodent hippocampus has been shown to contribute to the behavioural effects of SSRIs[16,17]. Such plastic changes could be the mechanism by which these drugs counteract the excessive excitability that is associated with anxiety disorders.

Gene–environment interactions and anxiety

Individuals seem to have a rather consistent level of trait anxiety over their lifetime[18–20], indicating that the degree of anxious behaviour persists over long periods and reflects fundamental differences in brain composition or wiring. Such differences in the brains of highly anxious versus less anxious individuals are likely to have developed as a result of differences in both the genetic makeup of individuals and the environment they have experienced during their life. Twin studies confirm this hypothesis. An analysis of the incidence of anxiety disorders in MONOZYGOTIC and DIZYGOTIC twins revealed that approximately 30–40% of the variance in occurrence between individuals can be attributed to genetic variation[21]. So, the magnitude of the genetic contribution to anxiety disorders is relatively moderate and less than that for more heritable psychiatric disorders such as schizophrenia, or neurological disorders such as Huntington's disease[22,23] (FIG. 1).

AMYGDALA
A small almond-shaped structure, comprising 13 nuclei, buried in the anterior medial section of each temporal lobe.

MONOZYGOTIC
Twins that develop from a single fertilized egg cell through its division into two genetically identical parts.

DIZYGOTIC
Twins that develop during the same pregnancy as the result of two separate eggs being fertilized by two separate sperm.

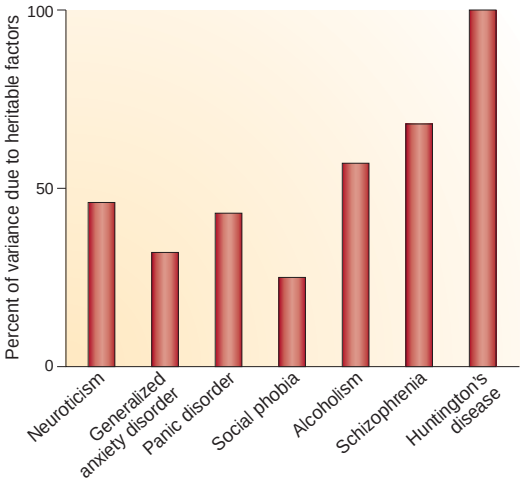

Figure 1 | **A comparison of the occurrence of mental illness in monozygotic and dizygotic twins reveals the influence of genetic factors.** For generalized anxiety disorder, panic disorder and social phobia, 30–40% of the variance in occurrence between individuals can be attributed to genetic factors. Trait anxiety, represented by neuroticism, is influenced by genetic factors to a similar degree, whereas genetic contributions to the aetiology of alcoholism, schizophrenia and Huntington's disease are greater.

Because it is very difficult to control for differences in an individual's environment, estimating the impact of environmental factors on the incidence of a phenotypic trait is problematic. However, by assuming that twins raised together are exposed to significantly similar familial environmental factors, estimates of the influence of shared environments on the prevalence of anxiety disorders have been calculated[21]. Surprisingly, these estimates are low, accounting for only about 5% of the variation in incidence of anxiety disorders. The apparently minor contribution of shared environment could be due to twins experiencing shared environments differently. In addition, both shared and individual-specific experiences are likely to be modified by, or dependent on, genetic factors (gene–environment interaction) or to be the product of genetic factors (gene–environment correlation). Gene–environment interactions and correlations are probably particularly important in illnesses with modest genetic components, such as anxiety disorders.

Only a small number of genetic variations have been linked to increased anxiety in humans. In several studies, a small but significant increase in anxiety was evident in both infants and adults that carry a variant of the 5-HT transporter (*5-HTT*) gene[24–26] (reviewed in REF. 27). The *5-HTT* gene promoter contains a simple repeat sequence — approximately 32% of the Caucasian population carry two short (*s*) alleles (14 repeats), 49% carry one short and one long (*l*) allele (16 repeats), and 19% carry two long alleles[24]. Homozygous *s*/*s* individuals have decreased cellular 5-HTT activity, and score higher for NEUROTICISM and lower for AGREEABLENESS on a personality inventory questionnaire than *s*/*l* or *l*/*l* individuals[24,25]. Similar increases in anxiety-related measures have also been documented for infants carrying the *s*/*s* combination[26]. These data indicate that this POLYMORPHISM has an important impact on early developmental events. Despite its small overall effect (it is estimated that the polymorphism accounts for less than 4% of the variance in this trait[24]), recent fMRI studies showed that the *s*/*s* allele combination is associated with increased activity of the amygdala during observation of fearful faces[28]. This finding indicates that 5-HTT influences anxiety-related behaviour by modulating the excitability of specific fear circuits in the brain.

These results seem to be at odds with the therapeutic efficacy of SSRIs, which block 5-HTT activity. However, the association between genetic impairment of 5-HTT function and increased anxiety is supported by studies of *5-HTT*-knockout mice, which exhibit increases in anxiety-related behaviour[29]. Intriguingly, this phenotype can be mimicked, at least partially, by pharmacological blockade of 5-HTT function during the first two weeks of life. This indicates that modulation of 5-HTT function during development can have the opposite effect on anxiety-related behaviours to modulation during adulthood[30].

PTSD is an example of an anxiety disorder in which environmental risk factors seem to be modulated by genetic factors. PTSD develops in approximately 15% of individuals that experience or witness severe trauma such as rape, murder or military combat. It is characterized by recurrent and intrusive memories of the traumatic event that elicit intense fear and severely disrupt normal life. One of the most consistent findings in the study of PTSD is a tendency for the volume of the hippocampus — a structure in the medial temporal lobe of the brain required for associative memory — to decrease[31]. The hippocampus is easily damaged by stress hormones[32,33], and several researchers have proposed that the decreased size of this brain region in PTSD patients is a direct consequence of the chronic state of stress that is induced by the trauma[34,35].

However, recent imaging studies of twins discordant for PTSD indicate that this hypothesis is incorrect[36]. These researchers propose that reduced hippocampal volume is a pre-existing condition that determines susceptibility to PTSD. They studied 40 pairs of monozygotic twins — one twin had experienced combat in Vietnam, while the other had stayed at home. Of those with combat experience, 42% developed PTSD. MRIs of the twins' brains showed that hippocampal volume did not differ significantly between those that developed PTSD and their stay-at-home siblings, supporting the assertion that the reduction in hippocampal volume associated with PTSD is not a consequence of the traumatic event or of the ensuing disorder. Most interesting, however, was the significant inverse correlation between hippocampal volume and the probability of developing PTSD after combat exposure. This correlation could explain why only some individuals that experience trauma go on to develop PTSD, and indicates that a small hippocampus increases an individual's susceptibility to environmental stress (FIG. 2).

NEUROTICISM
One of five domains of the NEO-Personality Inventory psychological assessment tool. Neuroticism comprises six facets: anxiety, depression, angry hostility, self-consciousness, impulsivity and vulnerability.

AGREEABLENESS
One of five domains of the NEO-Personality Inventory psychological assessment tool. Agreeableness comprises six facets: trust, straightforwardness, altruism, compliance, modesty and tender-mindedness.

POLYMORPHISM
Variation in DNA sequence between individuals.

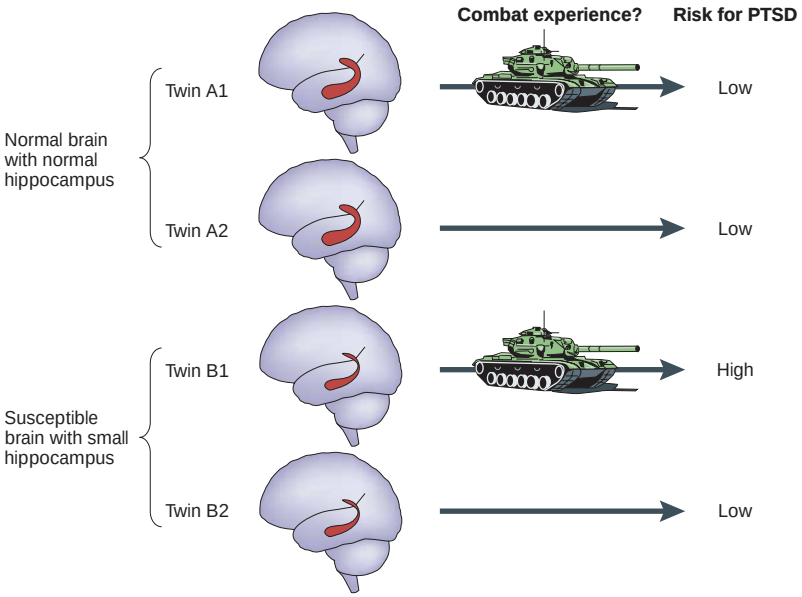

Figure 2 | **Post-traumatic stress disorder (PTSD) can develop as a result of a traumatic experience, such as military combat, and is associated with a reduction in hippocampal volume.** However, magnetic resonance imaging studies of monozygotic twins discordant for combat experience and PTSD show that combat-exposed twins have similar hippocampal volumes to their combat-naive siblings. In addition, lower hippocampal volume is associated with more severe PTSD, indicating that low hippocampal volume is a predisposing factor for PTSD rather than a consequence of the disease.

An important question left unanswered by these twin studies is whether the between-twin difference in hippocampal volume has a genetic or environmental origin. The strong correlation between the hippocampal volumes of monozygotic twins in this study indicates that genetic factors are important. But larger studies that compare monozygotic and dizygotic twins are necessary to determine relative genetic and environmental contributions. Presently, evidence from humans and rodents indicates that hippocampal volume is determined by both genetic and environmental factors[37–39]. In primates, hippocampal circuits are established mid-gestation and do not attain full maturity until adolescence. Hippocampal structure and function might be most susceptible to adverse influences during these developmental stages[40].

Developmental events and adult anxiety

A large body of data indicates that human susceptibility to psychopathology can be determined early in life. Psychologists have long supposed that early-life trauma increases the risk of psychiatric disorders developing subsequently. This hypothesis is supported by studies in which the number of severe early traumas suffered by patients was correlated with increased risk for adult disease pathology, including mood disorders[41,42]. For example, adults that had experienced four out of a list of seven severe early traumatic events had a 4.6-fold increased risk of developing depressive symptoms and were 12.2-fold more likely to attempt suicide[42]. No direct correlation between any specific childhood trauma and a specific adult anxiety disorder was evident, however, indicating that other, possibly genetic, factors determine the precise pathology that is precipitated by childhood trauma. In such a model, genetic risk factors for specific psychiatric disorders would depend on environmental influences acting early during the life of the individual.

Two particularly striking examples of such interactions in humans were recently discovered in longitudinal studies of children exposed to a violent family environment[43,44]. In the first study, severe early maltreatment was associated with a significantly increased risk of adolescent and adult antisocial behaviour, including conduct disorder, conviction for a violent offence and tendency to be violent. Furthermore, Caspi *et al.*[43] found that the impact of early maltreatment was strongly modified by a polymorphism in the promoter of the *MAOA* gene, which codes for an enzyme that metabolizes 5-HT, dopamine and noradrenaline. In boys carrying the low-activity allele of the *MAOA* gene, maltreatment is a significant risk factor for adolescent and adult antisocial behaviour, whereas maltreatment conferred no increased risk of antisocial behaviour on boys with the high-activity allele. This finding implies that the biochemical consequences of high *MAOA* activity are sufficient to protect the brain against the long-term consequences of childhood abuse.

In a second study using the same longitudinal cohort, rates of major depression at age 26 were found to be strongly influenced by both childhood abuse and the number of stressful life events in individuals carrying the *s*/*s* and *l*/*s* allele combinations of the 5-HTT promoter polymorphism, but not in those carrying the *l*/*l* combination[44]. Notably, predisposition to depression was not further modified by the *MAOA* polymorphism, indicating that there are different molecular mechanisms for MAOA- and 5-HTT-mediated susceptibilities. Given the high co-morbidity of depression and anxiety[45], and the evidence for their modulation by common genetic factors[46], it is likely that predisposition to anxiety disorders is also determined by developmental influences whose impact on the brain is under genetic control.

The observation that individuals are particularly susceptible to adverse environmental influences during early development was confirmed by animal studies that showed the powerful effects of the quality of maternal care on lifelong emotional behaviour and brain functioning. Replacement of the mother of an infant rhesus monkey with an inanimate surrogate during the first months of life induces long-term deficiencies in peer interaction and social adaptation. It is also associated with an increased risk of developing anxiety-related behaviours such as rocking and grooming[47,48]. Increasing the unpredictability associated with foraging for food causes bonnet macaque mothers to rear offspring that have abnormal stress hormone and fear responses in adulthood[49]. These studies indicate that early environmental trauma can directly induce long-term changes in the brain that alter fear and anxiety-related responses in adulthood. In rhesus monkeys, as in

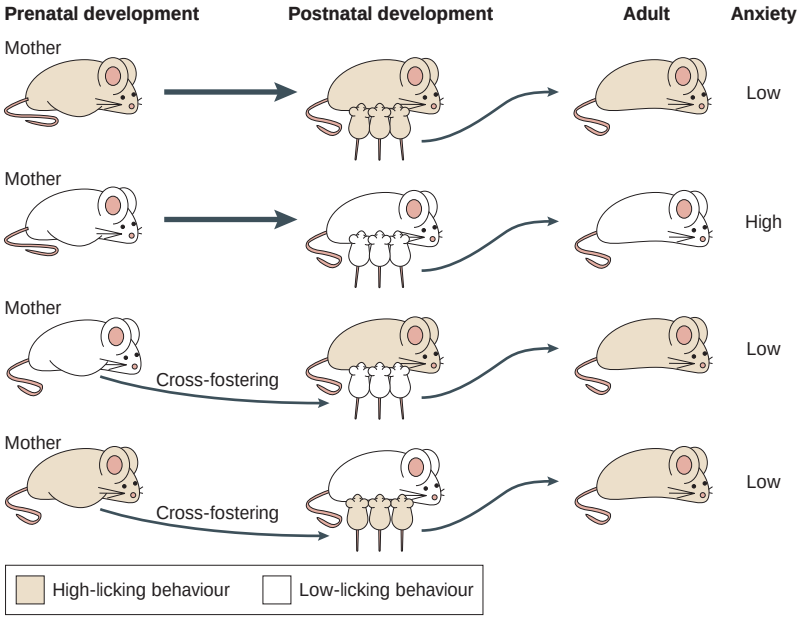

Figure 3 | **Rats raised by mothers that display low licking-and-grooming behaviour exhibit more anxiety-related behaviour than rats raised by high licking-and-grooming mothers.** Cross-fostering studies show that the offspring of low licking-and-grooming mothers raised by high licking-and-grooming mothers are less prone to anxiety-related behaviour as adults. This indicates that the effect is mediated by postnatal maternal environment. However, offspring of high licking-and-grooming mothers raised by low licking-and-grooming mothers do not have an increased tendency to develop anxiety-related behaviour in adulthood, indicating that specific factors inherited by the high licking-and-grooming offspring protect them from the effects of being mothered by low licking-and-grooming females.

humans, there are short and long versions of the 5-HTT promoter repeat[50]. As in humans, the short allele in monkeys is associated with increased levels of the 5-HT metabolite 5-HIAA, and an increase in anxiety-related behaviour[51]. Intriguingly, the effect of the 5-HTT polymorphism in monkeys is strongly modulated by early rearing environment. Monkeys reared by their mothers have normal levels of 5-HIAA regardless of *5-HTT* genotype. But monkeys reared in peer groups from 30 days to 7 months of age have significantly increased levels of 5-HIAA at maturity if they are of the *l*/*s* genotype, but not if they carry the *l*/*l* allele combination[52]. These data indicate that the physiological impact of the 5-HTT polymorphism is dependent on early maternal and social interactions.

Similarly, a number of studies have shown that, in rodents, maternal behaviour has long-lasting consequences for anxiety-related behaviour of the offspring. As adults, rats that were separated from their mothers for several hours a day during the early postnatal period are more likely to exhibit anxiety-related behaviours as well as increased hormonal reactivity to stress[53]. Pups raised by mothers with impaired licking and grooming skills have higher levels of anxiety-related behaviour than pups raised by high licking-and-grooming mothers[54]. Cross-fostering studies show that these influences are primarily environmental. Cross-fostering the offspring of low licking-and-grooming mothers to high licking-and-grooming mothers can decrease the risk of anxiety-related behaviour developing in the offspring[55]. However, the converse is not true. Offspring of high licking-and-grooming mothers raised by low licking-and-grooming mothers do not have an increased tendency to develop anxiety-related behaviour. This finding indicates that genetic or intra-uterine environmental factors imparted by high licking-and-grooming mothers confer protection against adverse effects of later mothering (FIG. 3). By transplanting embryos from a high-licking strain into low-licking surrogate mothers shortly after conception, Francis *et al.*[56] showed that the combination of consistent prenatal and postnatal maternal environments is sufficient to confer low-licking behaviour on the offspring of high-licking mice. So, intra- and extra-uterine maternal signals can synergistically induce long-term structural and functional changes in anxiety circuits.

Furthermore, Francis *et al.*[57] showed that experimentally conferred high licking-and-grooming behaviour can be passed from one generation to the next. Females raised by high licking-and-grooming mothers become high licking-and-grooming mothers themselves, and go on to produce low-anxiety offspring, regardless of whether their biological mother was of a low or high licking-and-grooming strain. This epigenetic inheritance of anxiety-related behaviour underscores the influence that environmental factors can exert to persistently remodel circuits in the brain during the early developmental period.

What molecular mechanisms are involved?

We know little about the molecular mechanisms by which early environmental influences alter anxiety circuits in the brain. Rats raised by high licking-and-grooming mothers have elevated levels of GLUCOCORTICOID receptor, brain-derived neurotrophic factor (BDNF), cyclic-AMP responsive element binding protein (CREB), acetylcholine esterase, and the synaptic marker synaptophysin, in the cortex and hippocampus[55,58]. The mechanism by which changes in the concentrations of these molecules persist into adulthood after maternal care ceases is not known. It has been suggested that long-term changes in transcription of the glucocorticoid receptor could be mediated by changes in the METHYLATION status of the gene[59].

Studies in genetically modified mice have made it possible to investigate the anxiety-related consequences of manipulating specific genes. A number of mouse strains in which mutations in specific genes have been induced (including knockout, knock-in and transgenic mice) show altered anxiety-related behaviour (reviewed in REFS 60,61). A defect in the establishment of brain circuits during development has been implicated in increased anxiety in at least one strain of knockout mouse. Mutation of the serotonin 1A (5-HT_{1A}) receptor in mice causes increases in anxiety-related behaviour[62–64]. This defect can be rescued by expression of the receptor in the forebrain under the control of calcium/calmodulin-dependent protein kinase IIα (CaMKIIα) regulatory sequences via the doxycycline-repressible transactivation system[65]. This conditional knockout

GLUCOCORTICOID
Hormones produced by the adrenal cortex, which are involved in carbohydrate and protein metabolism, but also affect brain function. Cortisol (human) and corticosterone (rodent) are examples.

METHYLATION
A chemical modification of a molecule involving the covalent attachment of a CH_3 group. Methylation of the DNA encoding a gene can alter its expression.

OCULAR DOMINANCE
In the mature primary visual cortex of mammals, most neurons respond predominantly to visual inputs from one eye or the other. Cells that respond to a given eye are arranged in stripes — the ocular dominance columns — that alternate with stripes of neurons that respond to the other eye.

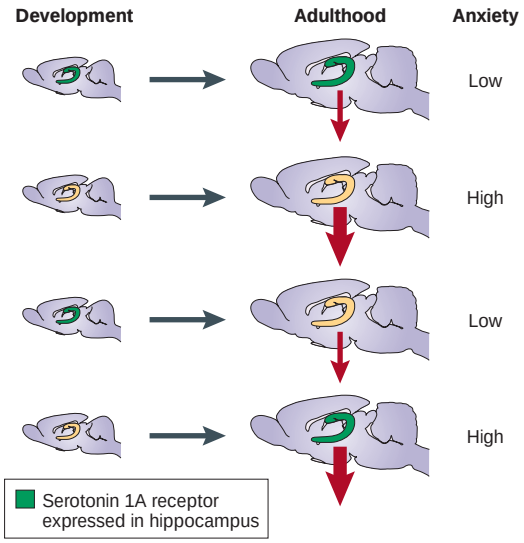

Figure 4 | **Serotonin receptor espression and anxiety.** In developing mice, expression of the serotonin 1A receptor in the forebrain is both necessary and sufficient to ensure normal anxiety-related behaviour later in life, regardless of whether the receptor is expressed during adulthood.

strategy was used to show that, whereas repression of receptor expression in the adult is ineffective, repression of receptor expression until four weeks of age is sufficient to produce adult mice with increased anxiety-related behaviour. This finding indicates that 5-HT is essential to the establishment of normal anxiety-modulating circuits in the brain during postnatal development (FIG. 4).

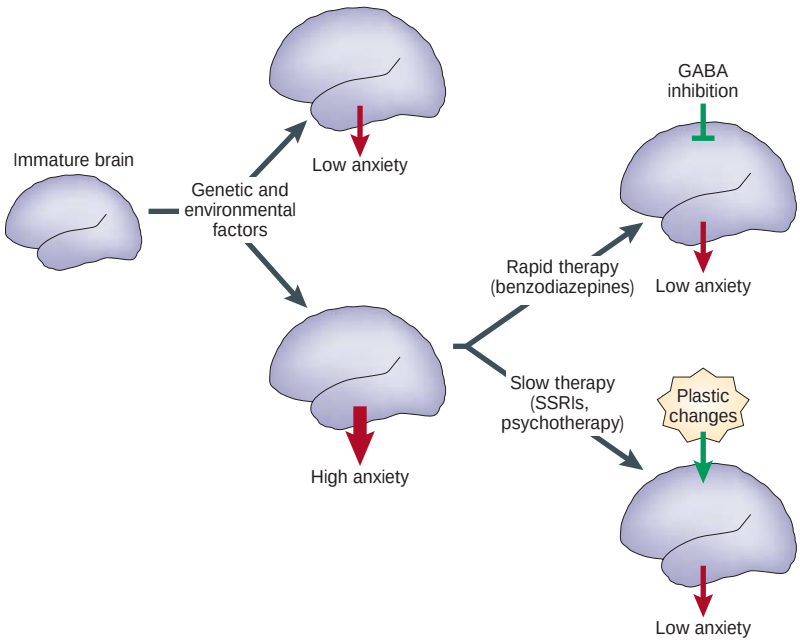

Figure 5 | **During development, genetic and environmental influences interact to modulate neuronal maturation and determine levels of anxiety.** In adulthood, high anxiety can be overcome by either rapid pharmacological treatment that directly blocks excessive excitability, or by slow pharmacological or psychotherapeutic treatments that induce compensatory plastic changes in the brain. GABA, γ-aminobutyric acid; SSRI, selective serotonin re-uptake inhibitor.

As expression of the receptor in the forebrain of 'rescued' mice was detectable only after the second postnatal week, the crucial period for establishment of the knockout phenotype is probably the third and fourth postnatal weeks, a period of dramatic synaptogenesis and dendritic growth in the forebrain. These results are supported by behavioural data which show that the anxiety-related phenotype of the knockout mice first appears at three weeks of age (C.G., unpublished data). Furthermore, dendritic branching and neuronal excitability are increased in the CA1 region of the hippocampus of mice that lack the 5-HT_{1A} receptor (J. Monckton and J.-P. Hornung, personal communication). This region has been shown to be important for regulating the innate anxiety-related behaviours that are abnormal in 5-HT_{1A}-receptor-knockout mice[66,67]. Maturation of dendritic branches in the CA1 region of the hippocampus occurs during the second, third and fourth weeks after birth, and overlaps with the sensitive period of 5-HT_{1A} receptor function[68]. It is interesting to speculate that this period of active synaptic development is a particularly crucial time for the adjustment of anxiety circuits in response to experience-dependent signals. Recent association studies in humans have found correlations between a functional single nucleotide polymorphism in the promoter of the 5-HT_{1A} receptor and both trait anxiety[69] and depression[70]. So, the 5-HT_{1A} receptor probably also modulates anxiety circuits in humans.

The molecular mechanisms that govern the susceptibility of developing synapses to environmental influences have been well studied in other brain systems. In the visual system, for example, monocular deprivation during early postnatal development induces a synaptic rearrangement called OCULAR DOMINANCE plasticity (reviewed in REF. 71). Neuronal excitability in the visual cortex — which can be under genetic as well as pharmacological control — determines susceptibility to ocular dominance plasticity[72]. Similarly, in the developing rodent somatosensory cortex, various factors, including autophosphorylation of CaMKIIα, modulate synaptic plasticity in response to competition between adjacent whisker inputs[73] (reviewed in REF. 74; see also REF. 75). We propose that similar molecular mechanisms might operate in developing limbic brain regions to integrate the effects of genetic factors — such as mutations in genes encoding the 5-HTT or 5-HT_{1A} receptor — and environmental factors, such as adverse early-life events.

In summary, lifelong susceptibility to anxiety can be determined by the combined influence of genetic and environmental factors during early development. Studies in humans, monkeys and rodents have revealed the importance of interactions between genetic and environmental factors in determining susceptibility to anxiety-related behaviour. In several recent human studies, early environmental risk factors for adult psychopathology have been shown to depend on the presence of specific genetic variations. Although early gene–environment interactions that affect the risk of developing anxiety disorders have not yet been

identified in humans, work with primates and rodents clearly shows the importance of such interactions in the aetiology of anxiety-related behaviours. Anxiety circuits might be particularly vulnerable to these factors during developmental periods when synaptic connections are elaborated and refined, and when brain circuits are highly plastic. Nevertheless, the efficacy of both psychotherapy and pharmacotherapy with SSRIs later in life indicates that anxiety circuits retain their plasticity in adulthood (FIG. 5). Understanding the molecular mechanisms that underlie the long-term impact of genetic and environmental factors on anxiety will help identify risk factors for these disorders and provide insight into natural variations in anxiety-related behaviour.

1. *Diagnostic and Statistical Manual of Mental Disorders* 4th edn (American Psychiatric Association, Washington DC, 1994).
2. Greenberg, P. E. *et al.* The economic burden of anxiety disorders in the 1990s. *J. Clin. Psychiatry* **60**, 427–435 (1999).
3. Nemeroff, C. B. Anxiolytics: past, present, and future agents. *J. Clin. Psychiatry* **64** (Suppl. 3), 3–6 (2003).
4. Martin, I. L. The benzodiazepines and their receptors: 25 years of progress. *Neuropharmacology* **26**, 957–970 (1987).
5. McNaughton, N. Cognitive dysfunction resulting from hippocampal hyperactivity — a possible cause of anxiety disorder? *Pharmacol. Biochem. Behav.* **56**, 603–611 (1997).
6. Osuch, E. A. *et al.* Regional cerebral metabolism associated with anxiety symptoms in affective disorder patients. *Biol. Psychiatry* **48**, 1020–1023 (2000).
7. Davidson, R. J., Abercrombie, H., Nitschke, J. B. & Putnam, K. Regional brain function, emotion and disorders of emotion. *Curr. Opin. Neurobiol.* **9**, 228–234 (1999).
8. Hay, P. *et al.* Treatment of obsessive–compulsive disorder by psychosurgery. *Acta Psychiatr. Scand.* **87**, 197–207 (1993).
9. Low, K. *et al.* Molecular and neuronal substrate for the selective attenuation of anxiety. *Science* **290**, 131–134 (2000).
10. Elliot, M. J., Heal, D. J. & Marsden, C. A. *Experimental Approaches to Anxiety and Depression* (John Wiley & Sons, Hoboken, 1992).
11. Kent, J. M., Coplan, J. D. & Gorman, J. M. Clinical utility of the selective serotonin reuptake inhibitors in the spectrum of anxiety. *Biol. Psychiatry* **44**, 812–824 (1998).
12. Smith, T. D., Kuczenski, R., George-Friedman, K., Malley, J. D. & Foote, S. L. *In vivo* microdialysis assessment of extracellular serotonin and dopamine levels in awake monkeys during sustained fluoxetine administration. *Synapse* **38**, 460–470 (2000).
13. Davidson, R. J., Irwin, W., Anderle, M. J. & Kalin, N. H. The neural substrates of affective processing in depressed patients treated with venlafaxine. *Am. J. Psychiatry* **160**, 64–75 (2003).
14. Duman, R. S., Heninger, G. R. & Nestler, E. J. A molecular and cellular theory of depression. *Arch. Gen. Psychiatry* **54**, 597–606 (1997).
15. Chaput, Y., de Montigny, C. & Blier, P. Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: electrophysiological studies in the rat brain. *Naunyn Schmiedebergs Arch. Pharmacol.* **333**, 342–348 (1986).
16. Malberg, J. E., Eisch, A. J., Nestler, E. J. & Duman, R. S. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J. Neurosci.* **20**, 9104–9110 (2000).
17. Santarelli, L. *et al.* Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301**, 805–809 (2003).
18. Kagan, J. & Snidman, N. Early childhood predictors of adult anxiety disorders. *Biol. Psychiatry* **46**, 1536–1541 (1999).
19. Schwartz, C. E., Snidman, N. & Kagan, J. Adolescent social anxiety as an outcome of inhibited temperament in childhood. *J. Am. Acad. Child Adolesc. Psychiatry* **38**, 1008–1015 (1999).
A longitudinal study showing that early behavioural inhibition is a risk factor for adolescent social phobia. This work supports the argument that some anxiety disorders are preceded by specific early behavioural traits.
20. Van Ameringen, M., Mancini, C. & Oakman, J. M. The relationship of behavioral inhibition and shyness to anxiety disorder. *J. Nerv. Ment. Dis.* **186**, 425–431 (1998).
21. Hettema, J. M., Neale, M. C. & Kendler, K. S. A review and meta-analysis of the genetic epidemiology of anxiety disorders. *Am. J. Psychiatry* **158**, 1568–1578 (2001).
22. Cannon, T. D., Kaprio, J., Lonnqvist, J., Huttunen, M. & Koskenvuo, M. The genetic epidemiology of schizophrenia in a Finnish twin cohort. A population-based modeling study. *Arch. Gen. Psychiatry* **55**, 67–74 (1998).
23. Brinkman, R. R., Mezei, M. M., Theilmann, J., Almqvist, E. & Hayden, M. R. The likelihood of being affected with Huntington disease by a particular age, for a specific CAG size. *Am. J. Hum. Genet.* **60**, 1202–1210 (1997).
24. Lesch, K. P. *et al.* Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* **274**, 1527–1531 (1996).
The first study to associate a polymorphism in the *5-HTT* gene with anxiety traits in a healthy population.
25. Greenberg, B. D. *et al.* Association between the serotonin transporter promoter polymorphism and personality traits in a primarily female population sample. *Am. J. Med. Genet.* **96**, 202–216 (2000).
26. Auerbach, J. *et al.* Dopamine D4 receptor (D4DR) and serotonin transporter promoter (5-HTTLPR) polymorphisms in the determination of temperament in 2-month-old infants. *Mol. Psychiatry* **4**, 369–373 (1999).
A paper showing that infants homozygous for the short allele of the 5-HTT promoter polymorphism have increased negative emotionality and distress. These findings indicate that 5-HTT function has an impact on anxiety-related behaviours from an early age.
27. Lesch, K. P. Molecular foundation of anxiety disorders. *J. Neural Transm.* **108**, 717–746 (2001).
28. Hariri, A. R. *et al.* Serotonin transporter genetic variation and the response of the human amygdala. *Science* **297**, 400–403 (2002).
29. Holmes, A., Murphy, D. L. & Crawley, J. N. Abnormal behavioral phenotypes of serotonin transporter knockout mice: parallels with human anxiety and depression. *Biol. Psychiatry* **54**, 953–959 (2003).
30. Ansorge, M., Zhou, M., Lira, A. & Gingrich, J. A. Progressive motor deficits in mice lacking the serotonin transporter. *Soc. Neurosci. Abstr.* **337.6** (2003).
31. Bremner, J. D. Neuroimaging studies in post-traumatic stress disorder. *Curr. Psychiatry Rep.* **4**, 254–263 (2002).
32. Reagan, L. P. & McEwen, B. S. Controversies surrounding glucocorticoid-mediated cell death in the hippocampus. *J. Chem. Neuroanat.* **13**, 149–167 (1997).
33. Sapolsky, R. M. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch. Gen. Psychiatry* **57**, 925–935 (2000).
34. Sapolsky, R. M. Why stress is bad for your brain. *Science* **273**, 749–750 (1996).
35. Bremner, J. D. Alterations in brain structure and function associated with post-traumatic stress disorder. *Semin. Clin. Neuropsychiatry* **4**, 249–255 (1999).
36. Gilbertson, M. W. *et al.* Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nature Neurosci.* **5**, 1242–1247 (2002).
Research showing that hippocampal volume probably predicts susceptibility to develop PTSD following combat trauma, rather than being a consequence of the trauma.
37. Maguire, E. A. *et al.* Navigation-related structural change in the hippocampi of taxi drivers. *Proc. Natl Acad. Sci. USA* **97**, 4398–4403 (2000).
38. Narr, K. L. *et al.* A twin study of genetic contributions to hippocampal morphology in schizophrenia. *Neurobiol. Dis.* **11**, 83–95 (2002).
39. Lu, L., Airey, D. C. & Williams, R. W. Complex trait analysis of the hippocampus: mapping and biometric analysis of two novel gene loci with specific effects on hippocampal structure in mice. *J. Neurosci.* **21**, 3503–3514 (2001).
40. Khazipov, R. *et al.* Early development of neuronal activity in the primate hippocampus *in utero*. *J. Neurosci.* **21**, 9770–9781 (2001).
41. Tweed, J. L., Schoenbach, V. J., George, L. K. & Blazer, D. G. The effects of childhood parental death and divorce on six-month history of anxiety disorders. *Br. J. Psychiatry* **154**, 823–828 (1989).
42. Felitti, V. J. *et al.* Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study. *Am. J. Prev. Med.* **14**, 245–258 (1998).
A large review of hospital admissions data showing a correlation between reported childhood trauma and adult pathology, including psychiatric disorders.
43. Caspi, A. *et al.* Role of genotype in the cycle of violence in maltreated children. *Science* **297**, 851–854 (2002).
44. Caspi, A. *et al.* Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* **301**, 386–389 (2003).
A longitudinal population study showing that a gene–environment interaction between probable assessments of early childhood maltreatment or life stressors and the short 5-HTT promoter polymorphism influences the incidence of depression in adulthood. This was the first study to implicate a specific gene–environment interaction in the aetiology of mood disorders.
45. Kaufman, J. & Charney, D. Comorbidity of mood and anxiety disorders. *Depress. Anxiety* **12** (Suppl. 1), 69–76 (2000).
46. Kendler, K. S. Major depression and generalised anxiety disorder. Same genes, (partly) different environments — revisited. *Br. J. Psychiatry* (Suppl.) 68–75 (1996).
47. Harlow, H. F., Dodsworth, R. O. & Harlow, M. K. Total social isolation in monkeys. *Proc. Natl Acad. Sci. USA* **54**, 90–97 (1965).
48. Ruppenthal, G. C., Arling, G. L., Harlow, H. F., Sackett, G. P. & Suomi, S. J. A 10-year perspective of motherless-mother monkey behavior. *J. Abnorm. Psychol.* **85**, 341–349 (1976).
49. Rosenblum, L. A., Forger, C., Noland, S., Trost, R. C. & Coplan, J. D. Response of adolescent bonnet macaques to an acute fear stimulus as a function of early rearing conditions. *Dev. Psychobiol.* **39**, 40–45 (2001).
50. Lesch, K. P. *et al.* The 5-HT transporter gene-linked polymorphic region (5-HTTLPR) in evolutionary perspective: alternative biallelic variation in rhesus monkeys. *J. Neural Transm.* **104**, 1259–1266 (1997).
51. Champoux, M. *et al.* Serotonin transporter gene polymorphism, differential early rearing, and behavior in rhesus monkey neonates. *Mol. Psychiatry* **7**, 1058–1063 (2002).
A study showing the contribution of a gene–environment interaction between levels of early maternal care and a 5-HTT promoter polymorphism to the anxiety-related behaviour of infant monkeys.
52. Bennett, A. J. *et al.* Early experience and serotonin transporter gene variation interact to influence primate CNS function. *Mol. Psychiatry* **7**, 118–122 (2002).
53. Kalinichev, M., Easterling, K. W., Plotsky, P. M. & Holtzman, S. G. Long-lasting changes in stress-induced corticosterone response and anxiety-like behaviors as a consequence of neonatal maternal separation in Long-Evans rats. *Pharmacol. Biochem. Behav.* **73**, 131–140 (2002).
54. Caldji, C. *et al.* Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proc. Natl Acad. Sci. USA* **95**, 5335–5340 (1998).
This research revealed a correlation between levels of maternal care and anxiety-related behaviour of adult rats.
55. Liu, D., Diorio, J., Day, J. C., Francis, D. D. & Meaney, M. J. Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nature Neurosci.* **3**, 799–806 (2000).
56. Francis, D. D., Szegda, K., Campbell, G., Martin, W. D. & Insel, T. R. Epigenetic sources of behavioral differences in mice. *Nature Neurosci.* **6**, 445–446 (2003).
57. Francis, D., Diorio, J., Liu, D. & Meaney, M. J. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science* **286**, 1155–1158 (1999).
58. Weaver, I. C. *et al.* Early environmental regulation of hippocampal glucocorticoid receptor gene expression: characterization of intracellular mediators and potential genomic target sites. *Mol. Cell. Endocrinol.* **185**, 205–218 (2001).

59. Weaver, I. C., Szyf, M. & Meaney, M. J. From maternal care to gene expression: DNA methylation and the maternal programming of stress responses. *Endocr. Res.* **28**, 699 (2002).
60. Holmes, A. Targeted gene mutation approaches to the study of anxiety-like behavior in mice. *Neurosci. Biobehav. Rev.* **25**, 261–273 (2001).
61. Finn, D. A., Rutledge-Gorman, M. T. & Crabbe, J. C. Genetic animal models of anxiety. *Neurogenetics* **4**, 109–135 (2003).
62. Parks, C. L., Robinson, P. S., Sibille, E., Shenk, T. & Toth, M. Increased anxiety of mice lacking the serotonin1A receptor. *Proc. Natl Acad. Sci. USA* **95**, 10734–10739 (1998).
63. Ramboz, S. *et al.* Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc. Natl Acad. Sci. USA* **95**, 14476–14481 (1998).
64. Heisler, L. K. *et al.* Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. *Proc. Natl Acad. Sci. USA* **95**, 15049–15054 (1998).
65. Gross, C. *et al.* Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature* **416**, 396–400 (2002).
This paper showed a requirement for early serotonin function in setting adult levels of anxiety-related behaviour in mice.
66. Deacon, R. M., Bannerman, D. M. & Rawlins, J. N. Anxiolytic effects of cytotoxic hippocampal lesions in rats. *Behav. Neurosci.* **116**, 494–497 (2002).
67. Kjelstrup, K. G. *et al.* Reduced fear expression after lesions of the ventral hippocampus. *Proc. Natl Acad. Sci. USA* **99**, 10825–10830 (2002).
68. Pokorny, J. & Yamamoto, T. Postnatal ontogenesis of hippocampal CA1 area in rats. II. Development of ultrastructure in stratum lacunosum and moleculare. *Brain Res. Bull.* **7**, 121–130 (1981).
69. Strobel, A. *et al.* Allelic variation in 5-HT_{1A} receptor expression is associated with anxiety- and depression-related personality traits. *J. Neural Transm.* **110**, 1445–1453 (2003).
70. Lemonde, S. *et al.* Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. *J. Neurosci.* **23**, 8788–8799 (2003).
71. Crowley, J. C. & Katz, L. C. Ocular dominance development revisited. *Curr. Opin. Neurobiol.* **12**, 104–109 (2002).
72. Fagiolini, M. & Hensch, T. K. Inhibitory threshold for critical-period activation in primary visual cortex. *Nature* **404**, 183–186 (2000).
This article describes an important role for a gene–environment interaction between early monocular deprivation and levels of GABA in ocular dominance plasticity in the developing mouse visual system.
73. Glazewski, S., Giese, K. P., Silva, A. & Fox, K. The role of α-CaMKII autophosphorylation in neocortical experience-dependent plasticity. *Nature Neurosci.* **3**, 911–918 (2000).
74. Gaspar, P., Cases, O. & Maroteaux, L. The developmental role of serotonin: news from mouse molecular genetics. *Nature Rev. Neurosci.* **4**, 1002–1012 (2003).
75. Whitaker-Azmitia, P. M., Druse, M., Walker, P. & Lauder, J. M. Serotonin as a developmental signal. *Behav. Brain Res.* **73**, 19–29 (1996).
76. Barlow, D. H. *Anxiety and its Disorders. The Nature and Treatment of Anxiety and Panic* 2nd edn (Gilford Press, New York, 2001).

Acknowledgements

We thank D. Leonardo, J. Gordon, A. Dranovsky, M. Rogan, K. Klemenhagen, J. Monckton, L. Santarelli and V. Carola for helpful comments and discussions.

Competing interests statement

The authors declare that they have no competing financial interests.

Online links

DATABASES

The following terms in this article are linked online to:

Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
BDNF | CREB | *5-HTT* | *MAOA*

OMIM:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
OCD | panic disorder

FURTHER INFORMATION

Gross laboratory homepage: http://www-db.embl-heidelberg.de/jss/emblGroups/g_177.html
Hen's homepage:
http://www.research.hs.columbia.edu/Faculty_Profiles/profiles/hen_r.html

Access to this interactive links box is free online.