Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 480 (2003) 185–204
www.elsevier.com/locate/ejphar

Anxiety-related traits in mice with modified genes of the serotonergic pathway

Klaus Peter Lesch*, Yong Zeng, Andreas Reif, Lise Gutknecht

Molecular and Clinical Psychobiology, Department of Psychiatry and Psychotherapy, University of Würzburg, Füchsleinstr. 15, 97080 Würzburg, Germany

Accepted 25 August 2003

Abstract

The neurobiology of anxiety is complex, reflecting the cumulative physiological effects of multiple genes. These genes are interactive with each other and with the environment in which they are expressed. Variation in genes coding for proteins that control serotonin (5-HT) system development and plasticity, establish 5-HT neuron identity, and modulate 5-HT receptor-mediated signal transduction and cellular pathways have been implicated in the genetics of anxiety and related disorders. Here, we selected anxiety and avoidance as paradigmatic traits and behavior and cover both traditional studies with inbred murine strains and selected lines which have been modified by gene knockout technologies. The design of a mouse model partially or completely lacking a gene of interest during all stages of development (constitutive knockout) or in a spatio-temporal context (conditional knockout) is among the prime strategies directed at elucidating the role of genetic factors in fear and anxiety. In many cases, knockout mice have been able to confirm what has already been anticipated based on pharmacological studies. In other instances, knockout studies have changed views of the relevance of 5-HT homeostasis in brain development and plasticity as well as processes underlying emotional behavior. In this review, we discuss the pertinent literature regarding phenotypic changes in mice bearing inactivation mutations of 5-HT receptors, 5-HT transporter, monoamine oxidase A and other components of the serotonergic pathway. Finally, we attempt to identify future directions of genetic manipulation in animal models to advance our understanding of brain dysregulation characteristic of anxiety disorders.

Keywords: Anxiety; Aggression; 5-HT (5-hydroxytryptamine, serotonin); Transgenics; Knockout mouse

1. Introduction

Behavioral, imaging, pharmacologic, and genetic studies indicate that anatomically and functionally distinct neural circuits as well as numerous neurotransmitters, growth factors, hormones, and their intracellular signaling effectors influence fear and anxiety in humans and animal models (Ninan, 1999). In humans, fear and anxiety represent internal emotional states and are natural adaptive consequences of stress that help to cope with the stressor. However, unlike the relatively mild, brief anxiety resulting from a stressful event such as an exam or public speaking, anxiety disorders are dysfunctional, chronic, persistent, and can grow progressively worse if not treated. Anxiety in rodents is defined as a high level of avoidance of novel and unfamiliar environment and increased fear reaction (Finn et al., 2003; Weiss et al., 2000). Other aspects of anxiety such as autonomic activation, increased stress reactivity, and neuroendocrine abnormalities have also been described. There are a number of experimental paradigms that have been used to detect and quantify these ‘‘anxiety-like’’ behaviors (Crawley, 1999; Crawley and Paylor, 1997). When rodents are introduced into a novel environment, they tend to move around the perimeter of the environment (‘‘open field’’). They stop occasionally and rear up, sniffing the walls and the floor. They spend very little time in the open center of the area, especially at first. If they have a choice, they will spend more time in a dark area than in a brightly lit one (‘‘light-dark Box’’). They will spend more time in a small, elevated area enclosed by walls than in an elevated area without walls (‘‘elevated plus maze’’). When they move from one delimited area into another, they often engage in a kind of stretching-out behavior. Anxiety-related traits often seem to be opposed to novelty-seeking behavior support-

* Corresponding author. Tel.: +49-931-201-77600; fax: +49-931-201-77620.
E-mail address: kplesch@mail.uni-wuerzburg.de (K.P. Lesch).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.08.106

ing the view that avoidance and curiosity share common biological underpinnings.

Behavioral genetics has persuasively demonstrated the relevance of both genetic and environmental factors for anxiety-related traits as well as anxiety disorders (Lesch, 2002; Lesch et al., 2002). It is no longer controversial whether nature or nurture shapes trait development but how complex genetic and environmental factor interact in the configuration of a behavioral phenotype. A complementary approach to genetic studies of anxiety and related disorders in humans involves investigation of genes and their protein products implicated in the brain neurocircuitry of fear and anxiety in animal models (Finn et al., 2003; Lesch, 2001a). An increasing body of evidence suggests that genetically driven variability of expression and function of proteins that regulate the function of brain neurotransmitter systems (e.g., receptors, ion channels, transporters, and enzymes) is associated with complex behavioral traits. Based on these findings, research is also giving strong emphasis to the molecular psychobiological basis of anxiety-related behaviors in rodents and, increasingly, non-human primates. Identification of molecular component of the neural circuits involved in fear and anxiety are currently leading to new candidate genes of presumed pathophysiologic pathways, in addition to candidates that are commonly derived hypothesized pathogenetic mechanisms of the disorder or from observations of therapeutic response.

Here, we describe fundamental aspects of the genetics of anxiety-related traits and responses and attempt an appraisal of quantitative genetic research in mice. This overview specifically focuses on behavioral and physiological consequences in mice with genetic manipulation of the serotonergic pathway and emphasizes its implications for the serotonin (5-HT) hypothesis of anxiety disorders as well as for novel anxiolytic drug development. Finally, we will discuss conceptual issues in the search for candidate genes for anxiety and for the development of mouse models of anxiety disorders.

2. Quantitative genetics of fear and anxiety

Quantitative genetic research on animal models consists primarily of inbred strain and selection studies. While comparisons between different inbred strains of mice expose remarkable differences in measures of anxiety-related behavior, such as performance in the open field or elevated plus maze paradigm, differences within strains can be attributed to environmental influences. Inbred and recombinant inbred strain studies are highly efficient in dissecting genetic influences, for investigating interactions between genotype and environment, and for testing the disposition-stress model (Eley and Plomin, 1997; Flint and Corley, 1996). In addition, embryo transfer and cross fostering have been employed to identify pre- and postnatal epigenetic mechanisms (Francis et al., 1999, 2003).

Selective breeding of mice for many generations produces difference between high and low anxiety lines that steadily increase each generation. Selection studies of behavioral traits strongly suggest a genetic influence and that many genes contribute to variation in behavior. Mice strains that have been selectively bred to display a phenotype of interest are currently being used to identify genetic loci that contribute to behavioral traits. The quantitative trait locus (QTL) approach has been applied with some success to a trait in mice called ‘‘emotionality’’ (Flint et al., 1995). Crosses between the high and low activity selected mouse lines yielded three QTL regions that appear to be related to various measures of fearfulness. A modified QTL strategy that uses recombinant inbred mouse strains produced candidate QTLs for open field fearfulness (Phillips et al., 1995).

Using the light–dark transition paradigm, Gershenfeld and Paul (1997) reported a significant QTL on chromosome 10 (near D10Mit237) and suggestive QTL were mapped to chromosomes 6, 15, 19, and X. For the open field test, QTL were identified on chromosome 1 and suggestive QTL were mapped to chromosomes 6 and 14. These QTL individually explain from 2.3% to 8.4% of the phenotypic variance and, when taken together, the multiple independent QTL represent 3.5% to 26.5% of the phenotypic variance, depending on the trait. The complexity and heterogeneity of the genetic factors underlying anxiety-like behaviors are illustrated by the lack of shared QTL between paradigms.

However, such linkage analyses provide only a rough chromosomal localization, whereas the next step, identifying the relevant genes by positional cloning, remains a challenging task (Tecott and Barondes, 1996). Since mice and humans share many orthologous genes mapped to synthenic chromosomal regions, it is conceivable that individual genes identified for one or more types of murine anxiety-related behavior may be developed as animal models for human anxiety. Following chromosomal mapping of polymorphic genes and evaluation of gene function using knockout (KO) mutants, behavioral parameters, including the type of anxiety, measure of anxiety, and test situation are investigated. Thus, the combination of elaborate genetic and behavioral analyses results in the identification of many genes with effects on variation and development of murine anxiety-related behavior and, ultimately, mouse QTL research is likely to generate candidate genes for human anxiety disorders (Eley and Plomin, 1997).

Recent advances in gene targeting (constitutive or conditional knockout techniques) are increasingly impacting upon our understanding of the neurobiologic basis of anxiety- and depression-related behavior in mice (Lesch, 2001a). However, the majority of neural substrates and circuitries that regulate emotional processes or cause anxiety disorders remain remarkably elusive. Among the reasons for the lack of progress are several conceptional deficiencies regarding the psychobiology of fear and anxiety, which make it difficult to develop and validate reliable models. The clinical presentation of anxiety disorders and the lack of

consensus on clinical categories further complicate the development of mouse models for specific anxiety disorders. In addition, human anxiety disorders encompass not only the behavioral trait of inappropriate fear but also the cognitive response towards this disposition. This response, however, is substantially modulated by environmental factors including cultural determinants, rearing, and sociocultural context. Investigations on the neurobiological basis of anxiety disorders therefore rely on the accurate dissection of behavioral dysfunctioning from other factors. The dilemma that no single paradigm mimics the diagnostic entities or treatment response of anxiety disorders may reflect that current classification systems are not based on the neurobiology of disease, rather than the failure to develop valid mouse models.

Various approaches have been employed to study anxiety-related traits in mice and the majority postulate that aversive stimuli, such as novelty or potentially harmful environments induce a central state of fear and defensive reactions, which can be assessed and quantified through physiologic and behavioral paradigms (Crawley, 1999; Crawley and Paylor, 1997). While substantial similarities between human and murine avoidance, defense or escape response exist, it remains obscure whether mice also experience subjective anxiety and associated cognitive processes similar to humans or whether defense responses represent pathological expression of anxiety in humans. In general, pathological anxiety may reflect an inappropriate activation of normally adaptive, evolutionarily conserved defense reaction. It should therefore be practicable to elucidate both physiologic and pathologic anxiety by studying avoidant and defensive behavior in mice using a broad range of anxiety models to ensure comprehensive characterization of the behavioral phenotype.

3. Serotonergic system and emotionality

A neural circuit composed of several regions of the prefrontal cortex, amygdala, hippocampus, medial preoptic area, hypothalamus, anterior cingulate cortex, insular cortex, ventral striatum, and other interconnected structures have been implicated in emotion regulation including the associated affective phenomena of fear and anxiety. Fear and anxiety-related circuits involve pathways transmitting information to and from the amygdala to various neural networks that control the expression of avoidant, defensive or aggressive reactions, including behavioral, autonomic, and stress hormone responses (Gorman et al., 2000). While pathways from the thalamus and cortex (sensory and prefrontal) project to the amygdala, inputs are processed within intra-amygdaloid circuitries and outputs are directed to the hippocampus, brain stem, hypothalamus, and other regions. Thus, the amygdala-associated neural network is critical to integration of the ‘‘fight or flight’’ response. Although the brain systems mediating anxiety-related responses appear to be fairly constant among mammals, several details of the regulatory pathways are species specific. While genetic and environmental factors contribute to the structure and function of this circuitry, the amygdala is central to processes of learning to associate stimuli with events that are either punishing or rewarding.

The function of the amygdala in emotion regulation is highly complex. Perception of danger or threat are transmitted to the lateral nucleus of the amygdala, which projects to the basal nuclei where information regarding the social context derived from orbitofrontal projections is integrated with the perceptual information. Behavioral responses can then be initiated via activation of projections from the basal nuclei to various association cortices, while physiological responses can be produced via projections from the basal nuclei to the central nucleus and then to the hypothalamus and brainstem. Excessive or insufficient activation of the amygdaloid complex leads to either disproportionate negative emotionality or impaired sensitivity to social signals. The orbitofrontal cortex, through its connections with other domains of the prefrontal cortex and with the amygdala, plays a critical role in limiting emotional outbursts, and the anterior cingulate cortex recruits other neural systems during arousal and other emotions. Functional or structural abnormalities in these regions or in their interconnections can modify emotionality and anxiety.

In humans, non-human primates, and other mammals, preclinical and clinical studies have accumulated substantial evidence that serotonergic signaling is a major modulator of emotional behavior including fear and anxiety, as well as aggression and integrates complex brain functions such as cognition, sensory processing, and motor activity. This diversity of these functions is due to the fact that 5-HT orchestrates the activity and interaction of several other neurotransmitter systems. The central 5-HT system, which originates in the midbrain and brainstem raphe complex, is widely distributed throughout the brain and its chemical messenger is viewed as a master control neurotransmitter within this highly elaborate system of neural communication mediated by 14+ pre- and postsynaptic receptor subtypes with a multitude of isoforms (e.g., functionally relevant splice variants) and subunits. The prefrontal cortex receives major serotonergic input, which appears dysfunctional in individuals who are emotionally unstable and stress reactive. Individuals vulnerable to faulty regulation of emotionality are therefore at risk for anxiety-related disorders.

The level of 5-HT in the synaptic (and extrasynaptic) space is restricted by the synchronized action of at least three components. Firing of raphe 5-HT neurons is controlled by 5-HT_{1A} autoreceptors located in the somatodendritic section of neurons. Release of 5-HT at the terminal fields is regulated by the 5-HT_{1B} receptor. Once released, 5-HT is taken up by the 5-HT transporter located at the terminals (and the somatodendritic section) of 5-HT neurons, where it is eventually metabolized by monoamine oxidase A. It was predicted that removal of one of these

components would affect extracellular levels of 5-HT. The action of 5-HT as a messenger is regulated by 5-HT synthesizing and metabolizing enzymes, and the 5-HT transporter. Serotonergic raphe neurons diffusely project to all brain regions implicated in anxiety-related behavior, while neurons in anxiety-mediating areas are rich in both 5-HT_1 and 5-HT_2 receptor subtypes. In addition to its role as a neurotransmitter, 5-HT is, via its receptors, an important regulator of morphogenetic activities during early brain development as well as during adult neurogenesis and plasticity, including cell proliferation, migration, differentiation, and synaptogenesis (Azmitia and Whitaker-Azmitia, 1997; Di Pino et al., in press; Lauder, 1993).

3.1. 5-HT receptors

The effects of 5-HT are mediated by at least 14 different 5-HT receptors. Pharmacological classification based on ligand binding experiments and on the study of functional responses to agonists/antagonists were initially utilized to define four 5-HT receptor subfamilies, 5-HT_{1-4}. Molecular biology has subsequently both confirmed this classification and also revealed the existence of novel 5-HT receptor subtypes for which little pharmacological or functional data exists (5-$HT_{1E/F}$, 5-$HT_{3A/B}$, 5-$HT_{5A/B}$, 5-HT_6, and 5-HT_7) (Barnes and Sharp, 1999; Hoyer and Martin, 1997). In 5-HT_{2-7} receptor genes, the coding region is interrupted by introns, whereas the genes for 5-HT_{1A-F} receptors contain no introns. The 5-HT_{2B}, 5-HT_4, 5-HT_6, and 5-HT_7 receptors are alternatively spliced and RNA editing of the 5-HT_{2C} receptor subtype in the second intracellular loop confers differential receptor functionality, thus increasing the complexity of the 5-HT receptor superfamily. The present challenge is to determine the physiological relevance of these gene products, establish their functionality as endogenous receptors, find selective ligands, and determine potential therapeutic application of these compounds.

The molecular characterization of different 5-HT receptor families has simplified the elucidation of gene transcription, mRNA processing and translation, as well as intracellular trafficking and posttranslational modification relevant to synaptic and postreceptor signaling. Transcriptional control regions have been cloned for several 5-HT receptor subtypes and functional promoter mapping data are available for the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_3 receptor genes. The analysis of genomic regulatory regions of 5-HT receptor genes and modeling variable 5-HT receptor gene function in genetically engineered mice (constitutive and conditional knockout/in) provides critical knowledge regarding the respective role of these receptors in neurodevelopment, synaptic plasticity, and behavior (Bonasera and Tecott, 2000).

3.1.1. 5-HT_{1A} receptor

The 5-HT_{1A} receptor subtype is implicated in the pathophysiology of anxiety and depression; its role as a molecular target of anxiolytic and antidepressant drugs is well established (Griebel, 1995; Griebel et al., 2000; Olivier et al., 1999). Patient with panic disorder and depression display an attenuation of 5-HT_{1A} receptor-mediated hypothermic and neuroendocrine responses, reflecting a reduced responsivity of both pre- and postsynaptic 5-HT_{1A} receptors (Lesch et al., 1990b, 1992). Likewise, a decrease in 5-HT_{1A} receptor ligand binding has been shown in postmortem brain of depressed suicide victims (Cheetham et al., 1990) as well as in forebrain areas such as the medial temporal lobe and in the raphe of depressed patients elicited by positron emission tomography (PET) (Drevets et al., 1999; Sargent et al., 2000). Both glucocorticoid administration and chronic stress, a pathogenetic factor in affective disorders, have also been demonstrated to result in the down-regulation of 5-HT_{1A} receptors in the hippocampus in the animals (Flugge, 1995; Lopez et al., 1998; Wissink et al., 2000). While deficits in hippocampal 5-HT_{1A} receptor function may contribute to the cognitive abnormalities associated with affective disorders, recent work suggests that activation of this receptors stimulates neurogenesis in the dentate gyrus (Nestler et al., 2002).

Downregulation and hyporesponsivity of 5-HT_{1A} receptors in patients with major depression is not reversed by the antidepressant drug treatment (Lesch et al., 1990a, 1991; Sargent et al., 2000), raising the possibility that low receptor function is a trait feature and therefore a pathogenetic mechanism of the disease. In line with this notion, evidence is accumulating that a polymorphism in the transcriptional control region of the 5-HT_{1A} receptor gene (HTR1A) resulting in allelic variation of 5-HT_{1A} receptor expression of is associated with personality traits of negative emotionality including anxiety and depression (Neuroticism and Harm Avoidance) as well as major depression, suicidality, and panic disorder (Rothe et al., in press; Strobel et al., in press).

5-HT_{1A} receptors operate both as somatodendritic autoreceptors and as postsynaptic receptors. Somatodendritic 5-HT_{1A} autoreceptors are predominantly located on 5-HT neurons and dendrites in the brainstem raphe complex and their activation by 5-HT or 5-HT_{1A} receptor agonists decreases the firing rate of serotonergic neurons and subsequently reduces the synthesis, turnover, and release of 5-HT from nerve terminals in projection areas. Postsynaptic 5-HT_{1A} receptors are widely distributed in forebrain regions that receive serotonergic input, notably in the cortex, hippocampus, septum, amygdala, and hypothalamus. Their activation results in neuronal inhibition, the consequences of which are not well understood, and in physiological responses that depend upon the function of the target cells (e.g. activation of the hypothalamic pituitary adrenocortical system) (Hamon et al., 1990). 5-HT_{1A} receptor expression is modulated by steroid hormones and 5-HT_{1A} receptor-mediated signaling is an important regulator of gene expression through its coupling to G proteins that inhibit adenylyl cyclase.

The effects of 5-HT_{1A} receptor selective agents, such as the agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT and the partial agonists ipsapirone and gepirone, have been extensively studied in rodents (De Vry, 1995). Both agonists and partial agonists induce a dose-dependent anxiolytic effect which correlates with the inhibition of serotonergic neuron firing, decrease of 5-HT release as well as the reduction of 5-HT signaling at postsynaptic target receptors. Blockade of the negative feedback by selective 5-HT_{1A} receptor antagonists, such as WAY 100635 increases firing of the serotonergic neurons but exerts no effect on 5-HT neurotransmission or behavior (Olivier and Miczek, 1999), while the combination with selective 5-HT reuptake inhibitors augments increases in 5-HT levels in terminal regions.

The converging lines of evidence that receptor deficiency or dysfunction is involved in mood and anxiety disorders encouraged investigators to genetically manipulate the 5-HT_{1A} receptor in mice. In 1998, a series of three papers reported the generation of mice with a targeted inactivation of the 5-HT_{1A} receptor (Heisler et al., 1998; Lesch and Mössner, 1999; Parks et al., 1998; Ramboz et al., 1998). Functional 5-HT_{1A} receptor knockout was confirmed by a complete lack of ligand binding to brain 5-HT_{1A} receptors in null-mutant (−/−) mice, with intermediate binding in the heterozygote (+/−) mice. Importantly, all three KO mouse strains display a similar behavioral phenotype characterized by increased anxiety-related behavior and stress reactivity in several different avoidance and behavioral despair paradigms.

3.1.1.1. Anxiety-related behavior. Mice with a targeted inactivation of the 5-HT_{1A} receptor consistently display a spontaneous phenotype that is associated with a gender-modulated and gene/dose-dependent increase of anxiety-related behaviors (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). With the exception of an enhanced sensitivity of terminal 5-HT_{1B} receptors, no major neuroadaptational changes were detected. Worthy of note is that this behavioral phenotype was observed in animals in which the mutation was bred into mice of Swiss-Webster (SW), C57BL/6J, and 129/SV backgrounds, consistently substantiating the assumption that this behavior is a authentic consequence of reduced or absent 5-HT_{1A} receptors. While all research groups used open field exploratory behavior as a model for assessing anxiety, two groups confirmed that 5-HT_{1A} receptor KO mice had increased anxiety by using other models, the elevated zero maze or elevated plus maze test (Heisler et al., 1998; Ramboz et al., 1998). These ethologically based conflict models test fear- and anxiety-related behaviors based on the natural tendencies for rodents to prefer enclosed, dark spaces versus their interest in exploring novel environments.

Activation of presynaptic 5-HT_{1A} receptors provide the brain with an autoinhibitory feedback system controlling 5-HT neurotransmission. Thus, enhanced anxiety-related behavior most likely represents a consequence of increased terminal 5-HT availability resulting from the lack or reduction in presynaptic somatodendritic 5-HT_{1A} autoreceptor negative feedback function (Lesch and Mössner, 1999). Although extracellular 5-HT concentrations and 5-HT turnover appear to be unchanged in the brain of 5-HT_{1A} receptor KO mice on the SW and 129SV backgrounds, indirect evidence for increased presynaptic serotonergic activity resulting in elevated synaptic 5-HT concentrations is provided by the compensatory upregulation of terminal 5-HT release-inhibiting 5-HT_{1B} receptors (Olivier et al., 2001; Toth, 2003). In contrast to 5-HT_{1A} receptor KO mice with a SW or 129/SV background, extracellular 5-HT concentrations were significantly elevated in mutant C57Bl6 mice in the frontal cortex ad hippocampus (Parsons et al., 2001). This may reflect a lack in compensatory changes in 5-HT_{1B} receptor and is consistent with findings that C57Bl6 mice are more aggressive and susceptible to drugs of abuse than many other strains.

Several studies addressed electrophysiological properties of both presynaptic serotonergic neurons and postsynaptic hippocampal neurons in 5-HT_{1A} receptor-deficient mice. A robust increase in the mean firing rate in dorsal raphe neurons was also reported, although a considerable number of neurons was firing in their normal range and 5-HT release was not altered (Richer et al., 2002). Moreover, mutant mice showed an absence of paired-pulse inhibition in the CA1 region and lack of paired-pulse facilitation in the dentate gyrus suggesting altered hippocampal excitability and impaired plasticity of the hippocampal network with consequence for cognition, learning, and memory (Sibille et al., 2000).

This mechanism is also consistent with recent theoretical models of fear and anxiety that are primarily based upon pharmacologically derived data. The cumulative reduction in serotonergic impulse flow to septohippocampal and other limbic and cortical areas involved in the control of anxiety is believed to explain the anxiolytic effects of ligands with selective affinity for the 5-HT_{1A} receptor in some animal models of anxiety-related behavior. This notion is based, in part, on evidence that 5-HT_{1A} receptor agonists (e.g. 8-OH-DPAT) and antagonists (e.g. WAY 100635) have anxiolytic or anxiogenic effects, respectively. However, to complicate matters further, 8-OH-DPAT has anxiolytic effects when injected in the raphe nucleus, whereas it is anxiogenic when applied to the hippocampus. Thus, stimulation of postsynaptic 5-HT_{1A} receptors has been proposed to elicit anxiogenic effects, while activation of 5-HT_{1A} autoreceptors is thought to induce anxiolytic effects via suppression of serotonergic neuronal firing resulting in attenuated 5-HT release in limbic terminal fields.

Since the 5-HT_{1A} receptor is expressed in different brain subsystems, it is of interest to clarify whether pre- or postsynaptic receptors are required to maintain normal expression of anxiety-related behavior in mice. With an elegant conditional rescue approach, Gross et al. (2002) showed that expression of the 5-HT_{1A} receptor in the hippocampus and

cortex but not in the raphe nuclei is required to rescue the behavioral phenotype of KO mice. The findings indicate that deletion of the 5-HT_{1A} receptor in mice, specifically in forebrain structures, results in a robust anxiety-related phenotype and that this phenotype in 5-HT_{1A} receptor KO mice is caused by the absence of the receptor during a critical period of postnatal development, whereas inactivation of 5-HT_{1A} receptors in adulthood does not affect anxiety. Even more importantly, the findings further support the notion of a central role for 5-HT in the early development of neurocircuit-mediating emotion (Di Pino et al., in press; Lesch, 2003). Although there is converging evidence that the 5-HT_{1A} receptor mediates anxiety-related behavior, the neurodevelopmental mechanism that renders 5-HT_{1A} receptor-deficient mice more anxious are highly complex and remain to be elucidated in its details.

While increased 5-HT availability and activation of other serotonergic receptor subtypes that have been shown to mediate anxiety (e.g. 5-HT_{2C} receptor) may contribute to increased anxiety in rodent models, multiple downstream neurotransmitter pathways or neurocircuits, including noradrenergic, γ-butyric acid (GABA)ergic, glutamatergic, and peptidergic transmission, as suggested by overexpression or targeted inactivation of critical genes within these systems (Lesch, 2001a), have been implicated to participate in the processing of this complex behavioral trait. Since avoidance induced by conflict and fear is only one dimension of anxiety-related responses, other components including autonomic systems activation, responsiveness to stress, 5-HT dynamics, and neuronal excitability in limbic circuitries appear to be involved in fear and anxiety.

3.1.1.2. Stress reactivity. As a facet of anxiety-like behavior, 5-HT_{1A} receptor KO mice show genotype-dependent and background strain-unrelated increase in stress reactivity in two paradigms of behavioral despair, the Forced Swim and Tail Suspension tests (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). Autonomic manifestations of anxiety and stress responsiveness in a novel environment or when exposed to other stressors increased heart rate and body temperature as well as attenuated release of corticosterone is also a characteristic of 5-HT_{1A} receptor KO mice (Groenink et al., 2003). The reduced immobility in stress/antidepressant test models is either due to an increased serotonergic tone resulting from the compromised 5-HT_{1A} autoreceptor-dependent negative feedback regulation or enhanced dopamine and norepinephrine function because it is reversed by pretreatment with α-methyl-*para*-tyrosine, but not by *para*-chlorophenylalanine (Mayorga et al., 2001).

Although the behavior of 5-HT_{1A} receptor-deficient mice in various stress-related paradigms is more consistent with increased emotionality, their behavior essentially corresponds with the performance of rodents treated with antidepressants. The role of 5-HT_{1A} receptors in the therapeutic action of antidepressant drugs has attracted extraordinary interest, however, there is substantial conflicting evidence regarding the involvement of other serotonergic receptor subtypes and neurotransmitter systems or neurocircuits that interact with 5-HT neurotransmission. Electrophysiological studies in rats indicate that each class of antidepressants enhances 5-HT neurotransmission via differential adaptive changes in the 5-HT_{1A} receptor-modulated negative feedback regulation that eventually leads to an overall increase of terminal 5-HT (for review, see Blier and de Montigny, 1998) and desensitization of 5-HT_{1A} receptor reponsivity following antidepressant treatment has been demonstrated in rodents (Le Poul et al., 1995; Li et al., 1993) and humans (Berlin et al., 1998; Sargent et al., 1997; Lesch et al., 1991; Lerer et al., 1999). While the neuroadaptive mechanism of antidepressant action of tricyclics or selective 5-HT reuptake inhibitors is exceedingly complex, as the onset of clinical improvement commonly takes 2–3 weeks or more after initiation of antidepressant drug administration, progressive functional desensitization of pre- and postsynaptic serotonergic receptors, including 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} receptors, that is set off by blockade of the 5-HT transporter, has been implicated in these delayed therapeutic effects. In conclusion, the phenotypic similarity between anxiety-related behavior and stress reactivity in humans and 5-HT_{1A} receptor KO mice validates the practicability of KO animal models.

3.1.2. 5-HT_{1B} receptor

The 5-HT_{1B} receptor was the first subtype to have its gene inactivated by classical homologous recombination (Saudou et al., 1994). 5-HT_{1B} receptors are expressed in the basal ganglia, central gray, lateral septum, hippocampus, amygdala, and raphe nuclei. They are located predominantly at presynaptic terminals inhibiting 5-HT release or, as heteroreceptors, modulating the release of other neurotransmitters. Selective agonists and antagonists for 5-HT_{1B} receptors are largely lacking, but indirect pharmacological evidence suggests that 5-HT_{1B} receptor activation influences food intake, sexual activity, locomotion, and emotionality including particularly impulsivity and aggression.

Generation of mice with a targeted disruption of the 5-HT_{1B} receptor gene facilitated investigation of the concept of 5-HT-related impulsivity in the context of aggressive behavior (Stark and Hen, 1999). Two of the behaviors, locomotion, and aggression, postulated to be modulated by 5-HT_{1B} receptors were analyzed. Wild-type and homozygous null mutant 5-HT_{1B} −/− mice were found to display similar levels of locomotor activity in an open field. However, 5-HT_{1B} receptor KO mice show adaptation in 5-HT_{2C} receptor-mediated functions with smaller reductions in food intake and locomotor activity in response to administration of 5-HT_{2C} receptor agonists. Impulsivity and aggression-related behavior of 5-HT_{1B} receptor −/− male mice was assessed by isolation and subsequent exposure to a non-isolated male wild-type intruder mouse. The latency and number of attacks displayed by the KO mice were used as indices of aggression. The 5-HT_{1B}

receptor KO mice, when compared with wild-type mice, showed more rapid, more intense, and more frequent attacks. Lactating female 5-HT_{1B} −/− receptor mice also attack unfamiliar male mice more rapidly and violently. In addition to increased aggression, KO mice acquire cocaine self-administration faster and ingest more ethanol than controls, indicating that the 5-HT_{1B} receptor not only modulates motor impulsivity and aggression but also addictive behavior (Brunner et al., 1999).

These results further support the notion that distinct receptor subtypes modulate different dimensions of behavior which may be either synergistic or antagonistic. Quite the opposite to 5-HT_{1A} receptor-deficient mice, 5-HT_{1B} receptor KOs are more reactive and more aggressive but show dramatically less anxiety-related behavior than control mice, although both 5-HT_{1A} and 5-HT_{1B} receptors control the tone of the serotonergic system and mediate some of the postsynaptic 5-HT effects (Zhuang et al., 1999). The regional variation of 5-HT receptor expression and the complex autoregulatory processes of 5-HT function which are operational in different brain areas may lead to a plausible hypothesis to explain a contradiction that is more apparent than real. Assessment of 5-HT_{1A} receptor expression in male mice selected for high and low offensive aggression showed that high-aggressive mice are characterized by a short attack latency, decreased plasma corticosterone concentration and increased levels of 5-HT_{1A} receptor mRNA in the dorsal hippocampus (dentate gyrus and CA1) compared to low-aggressive mice that had long attack latency and high plasma corticosterone levels (Korte et al., 1996). Increased postsynaptic 5-HT_{1A} receptor radioligand binding was found in the hippocampal CA1 subdivision, dentate gyrus, lateral septum, and frontal cortex, whereas no difference in ligand binding was found for the 5-HT_{1A} autoreceptor on cell bodies in the dorsal raphe nucleus. These results suggest that high offensive aggression is associated with reduced (circadian peak) plasma corticosterone and increased postsynaptic 5-HT_{1A} receptor availability in limbic and cortical regions.

3.1.3. Other 5-HT receptors

Prior to the generation of 5-HT_{2C} receptor-deficient mice, studies with nonselective agonists had suggested potential roles for this receptor in the serotonergic regulation of feeding and anxiety. Consistent with the pharmacological evidence, 5-HT_{2C} receptor mutant mice display not only hyperphagia-evoked weight gain but also infrequent and sporadic spontaneous seizures suggesting a globally enhanced neuronal network excitability. Behavioral analysis of 5-HT_{2C} receptor KO mice revealed abnormal performance in a spatial learning task and altered exploratory behavior, associated with altered long-term potentiation restricted to the dentate gyrus perforant path synapse (Heisler and Tecott, 1999). However, abnormalities of hippocampal function-dependent cognitive function were subtle and did not generalize to contextual fear conditioning.

Studies in mice with a targeted inactivation of other 5-HT receptor subtypes, such as the 5-HT_{5A} and 5-HT_7, or a transgenic line that overexpresses 5-HT_3 receptor, demonstrate that these receptors modulate the activity of neural circuits involved specifically in exploratory and reward-related behavior. When exposed to novel environments, KO mice lacking the 5-HT_{5A} receptor exhibit increased exploratory activity and an attenuated stimulatory effect of lysergic acid diethylamide (LSD) on exploratory activity but no change in anxiety-related behavior (Grailhe et al., 1999), whereas 5-HT_7 receptor KO mice do not express any overt behavioral phenotype at all (Hedlund et al., 2003).

Since all 5-HT receptor subtypes are remarkably similar in their ligand-binding domains, it has been difficult to design pharmacological compounds that will specifically interact with the other subtypes. The present challenge is therefore to further characterize the physiological and behavioral relevance of the remaining 5-HT receptor gene products and generate KO mice for each subtype. The new insights into neural plasticity and complexity of gene regulation in 5-HT subsystems will eventually provide the means for novel approaches of studying 5-HT receptor subtype-related behaviors at the molecular level.

3.2. 5-HT synthesizing and metabolizing enzymes

3.2.1. Tryptophan hydroxylases

The first step of 5-HT biosynthesis is catalyzed by the rate-limiting enzyme tryptophan hydroxylase (TPH). Two isoforms, TPH1 and TPH2, have been identified in the periphery and in 5-HT neurons, respectively. Both isoforms are members of the aromatic amino acid hydroxylase gene family, together with phenylalanine and tyrosine hydroxylases. The human TPH1 gene located on chromosome 11p15.1, spans a region of 30 kb, contains at least 11 exons, and an unusual splicing complexity in the 5′-untranslated region resulting in at least four TPH1 mRNA species transcribed from a single transcriptional start site (Boularand et al., 1995). The murine TPH1 has been mapped to chromosome 7 at 23.5 cM. The human TPH2 gene was found on chromosome 12p21.1, covers a region of more than 120 kb, and contains 11 exons, and a single TPH2 mRNA species is transcribed from a unique transcriptional start site (Walther et al., 2003). The murine TPH2 is located on chromosome 10. The deduced amino acid sequence of TPH2 shows 84% and 86% identity to TPH1 sequences of man, and mouse, respectively. Mice with an inactivation of the TPH1 gene lack 5-HT in the periphery, whereas 5-HT concentrations in serotonergic projection region of the brain are in the normal range. Moreover, 5-HT-related avoidance behavior as assessed by the elevated plus maze and hole board tests was not different in TPH1 KO mice indicating that the behavioral effects of 5-HT in the brain are uncoupled and thus independent from 5-HT and its metabolites in peripheral tissues.

This finding is critically relevant, since correlations between peripheral levels of 5-HT metabolites and 5-HT function in the brain of patients suffering from psychiatric disorders have extensively been studied. A possible role of TPH1 gene variations in antisocial personality disorder, alcoholism, bipolar disorder, major depression, and associated suicidality has been shown in some but not all studies (Bellivier et al., 1998; Furlong et al., 1998; Manuck et al., 1999; New et al., 1998). Nielsen et al. (1994, 1998) reported that the TPH A779C polymorphism influences 5-hydroxyindoleacetic acid concentrations (5-HIAA), the major metabolite of 5-HT, in cerebrospinal fluid (CSF), and may predispose to suicidality, a pathophysiological mechanism that may involve impaired impulse control. This finding was subsequently replicated by the several other groups suggesting that functional variant(s) in or close to the TPH1 gene may predispose individuals to affective disorders and suicidality.

In the face of preferential expression of TPH2 in the brain, the identification of a gene encoding a neuron-specific TPH isoform, and unaltered anxiety-like behavior in TPH1 KO mice this conclusion calls for reconsideration. Among the questions which need to be answered are whether TPH1 expression is restricted to early stages of brain development and whether peripheral 5-HT impacts on the development of limbic circuits setting the stage of emotional behavior and thus influencing susceptibility to anxiety, depression, and suicidality in adulthood. In anticipation of a mouse model with a targeted disruption of the TPH2 gene, clarification of the effect of brain 5-HT deficiency on anxiety-related and aggressive behavior seems to be imminent (Mallet, personal communication).

3.2.2. Monoamine oxidase A

Monoamine oxidase A (MAOA) oxidizes 5-HT, norepinephrine as well as dopamine, and is expressed in a cell type-specific manner. Abnormalities in MAOA activity have been implicated in a wide range of psychiatric disorders. Deficiency in MAOA enzyme activity due to a hemizygous chain termination mutation of the MAOA gene has recently been shown to be associated with impulsive aggression and hypersexual behavior in affected males from a single extended pedigree (Brunner syndrome) (Brunner et al., 1993).

Mice with a targeted disruption of the MAOA gene have markedly higher brain 5-HT concentrations and exhibit aggressive behavior in adult males as assessed by the resident–intruder paradigm as well as violent courtship (Seif and De Maeyer, 1999). Moreover, MAOA KO mice display reduced activity possibly reflecting increased anxiety in the open field and enhance stress reactivity in the forced swim test. Morphological analyses of brain structures where 5-HT has been suggested to act as a differentiation signal in development revealed a detrimental effect of MAOA inactivation on the formation and plasticity of cortical and subcortical structures. Investigations of 5-HT participation in neocortical development and plasticity have been concentrated on the rodent somatosensory cortex (SSC), due to its one-to-one correspondence between each whisker and its cortical barrel-like projection area. The processes underlying patterning of projections in the SSC have been intensively studied with a widely held view that the formation of somatotopic maps does not depend on neural activity. The timing of serotonergic innervation coincides with pronounced growth of the cortex, the period when incoming axons begin to establish synaptic interactions with target neurons and to elaborate a profuse branching pattern.

Interestingly, the brain of MAOA KO mice shows a lack of these characteristic barrel-like clustering of layer IV neurons in S1, despite relatively preserved trigeminal and thalamic patterns (Cases et al., 1995; Di Pino et al., in press). Thalamo-cortical afferents display a decrease in branching and excessive tangential distributions, suggesting a deficiency of terminal retraction (Rebsam et al., 2002) Other abnormalities include abnormal segregation of contralateral and ipsilateral retinogeniculate projections (Upton et al., 1999), and aberrant maturation of the brainstem respiratory network (Burnet et al., 2001). The excess 5-HT is likely responsible for these alterations, since barrel formation is restored by the 5-HT synthesis inhibitor *para*-chlorophenylalanine, which also restores normal development of retinogeniculate projections and the brainstem respiratory network as well as aggression-related behavior.

Additional evidence for a role of 5-HT in the development of neonatal rodent SSC derives from the transient barrel-like distribution of 5-HT, 5-HT_{1B} and 5-HT_{2A} receptors, and of the 5-HT transporter (Lebrand et al., 1996; Mansour-Robaey et al., 1998). The transient barrel-like 5-HT pattern visualized in layer IV of the SSC of neonatal rodents stems from 5-HT uptake and vesicular storage in thalamocortical neurons, transiently expressing at this developmental stage both 5-HT transporter and the vesicular monoamine transporter (VMAT2) despite their later glutamatergic phenotype (Lebrand et al., 1996).

3.3. Transporter

High-affinity 5-HT transport into the presynaptic neuron is mediated by a single protein, the 5-HT transporter (5-HTT, SERT), which is regarded as initial sites of action of antidepressant drugs and several neurotoxic compounds. Tricyclic antidepressants, such as prototypical imipramine, and the selective 5-HT uptake inhibitors, paroxetine, citalopram, and sertraline, occupy several pharmacologically distinct sites overlapping at least partially the substrate binding site and are widely used in the treatment of depression, anxiety, and impulse control disorders, as well as substance abuse including alcoholism.

While in adult brain, 5-HTT expression appears to be restricted to raphe neurons, it has been detected in the sensory areas of the cortex and thalamus during perinatal

development (Lesch and Murphy, in press). Cloning of the 5-HTT has identified a protein with 12-transmembrane domains and studies using site-directed mutagenesis and deletion mutants indicate that distinct amino acid residues participate in substrate translocation and competitive antagonist binding. 5-HTT function is acutely modulated by posttranslational modification. Moreover, several intracellular signal transduction pathways converge on the transcriptional apparatus of the 5-HTT gene regulating its expression. A polymorphism in the transcriptional control region of the human 5-HTT gene (SLC6A4) that results in allelic variation in functional 5-HTT expression is associated with anxiety, depression, and aggression-related personality traits (Lesch, 2003; Lesch et al., 1996). In addition to the exploration of the impact of allelic variation in 5-HTT expression on anxiety, depression, and aggression-related personality traits, a role of the regulatory and structural 5-HTT gene variation has been suggested in a variety of diseases such as depression, bipolar disorder, anxiety disorders, eating disorders, substance abuse, autism, schizophrenia, and neurodegenerative disorders (Lesch and Murphy, in press).

3.3.1. Anxiety-related responses

The converging evidence that 5-HTT deficiency plays a role in anxiety and related disorders lead to the generation of mice with a targeted inactivation of the 5-HTT gene (*Slc6a4*). Behavior of the 5-HTT KO mice was tested in a variety of conditions evaluating fear, avoidance, conflict, stress responsiveness, status of the neuroendocrine system, and effects of various pharmacological agents on the behavior. In particular, anxiety-related behaviors were characterized using a battery of tests including open field, elevated zero and plus maze, and light–dark box. In these tests, both male and female 5-HTT KO mice show consistently increased anxiety-like behavior and inhibited exploratory locomotion. The selective 5-HT_{1A} receptor antagonist WAY 100635 produced an anxiolytic effect in the elevated plus-maze in 5-HTT KO mice suggesting that the abnormalities in anxiety-like and exploratory behavior is mediated by the 5-HT_{1A} receptor. Unlike heterozygous 5-HT_{1A} +/− mice, 5-HTT +/− mice, in which transporter binding sites are reduced by approximately 50%, were similar to controls on most measures of anxiety-like behavior. However, changes in exploratory behavior in +/− mice were limited to specific measures under baseline conditions, but extended to additional measures under more stressful test conditions. This observation is in accordance with reduced aggressive behavior in 5-HTT +/− mice that is limited to specific measures and test conditions (Holmes et al., 2002). These subtle behavioral alterations in 5-HTT +/− mice are contrasted by robust perturbations in serotonergic homeostasis that are intermediate between −/− mice and controls in a gene/dose-dependent manner including elevated extracellular 5-HT, decreased 5-HT neuron firing in the dorsal raphe, and reduced 5-HT_{1A} receptor expression and function (Gobbi et al., 2001; Li et al., 1999, 2000). The evidence that serotonergic dysfunction in 5-HTT +/− mice may manifest and become noticeable as behavioral abnormalities only under challenging environmental conditions strongly support the disposition–stress model of affective and anxiety disorders.

3.3.2. Neuroadaptive changes

Analogous to 5-HT_{1A} KO mice, the neural mechanisms underlying increased anxiety-related behavior and reduced exploratory locomotion in mice with a disruption of the 5-HTT gene may relate to excess serotonergic neurotransmission which is expected to cause enhanced activation of postsynaptic 5-HT receptors. Both in vivo microdialysis in striatum and in vivo chronoamperometry in hippocampus revealed that 5-HTT null mutant mice exhibit an approximately 5-fold increase in extracellular concentrations of 5-HT and an absence of transporter-mediated clearance, although brain tissue 5-HT concentrations are markedly reduced by 40–60% (Bengel et al., 1998).

Excess of extracellular 5-HT activates the negative autoinhibitory feedback and reduces cellular 5-HT availability by stimulating 5-HT_{1A} receptors which results in their desensitization and downregulation in the midbrain raphe complex and, to a lesser extent, in hypothalamus, septum and amygdala but not in the frontal and hippocampus (Li et al., 2000). Although postsynaptic 5-HT_{1A} receptors appear to be unchanged in frontal cortex and hippocampus, indirect evidence for decreased presynaptic serotonergic activity but reduced 5-HT clearance resulting in elevated synaptic 5-HT concentrations is provided by compensatory alterations in 5-HT synthesis and turnover, downregulation of terminal 5-HT release-inhibiting 5-HT_{1B} receptors (Fabre et al., 1999).

Therefore, a partial downregulation of postsynaptic 5-HT_{1A} receptors in some forebrain regions but a several-fold increase in extracellular concentrations of 5-HT in 5-HTT null mutant mice could still cause excess net activation of postsynaptic 5-HT_{1A} receptors, resulting in increased anxiety-like behavior and its reversal by WAY 100635 (Holmes et al., in press). However, administration of WAY 100635 antagonizes not only postsynaptic 5-HT_{1A} receptors in forebrain regions but also acts at somatodendritic autoreceptors in the raphe nuclei and electrophysiological studies show that WAY 100635 causes a reversal of markedly reduced spontaneous firing rates of 5-HT neurons in the dorsal raphe dorsal nucleus of 5-HTT −/− mice, indicating that the net effect of WAY 100635 on serotonergic neurotransmission in 5-HTT KO mice may be more complex than anticipated (Gobbi et al., 2001).

Taken together these findings, add to an emerging picture of abnormalities in 5-HTT null mutants across a range of behavioral, neuroendocrine and physiological parameters associated with emotional disorders, including marked increases in adrenocorticotropin (ACTH) concentrations in responses to stress (Li et al., 1999), increased sensitivity to drugs of abuse such as cocaine (Sora et al., 1998, 2001),

altered gastrointestinal motility (Chen et al., 2001), and disturbed rapid eye movement sleep (Wisor et al., 2003). Finally, given the absence of the 5-HTT throughout ontogeny, 5-HTT KO mice also provide a research tool for studying the potential for neurodevelopment abnormalities affecting anxiety-like behavior.

3.3.3. Development of the somatosensory cortex

Analogous to MAOA KO mice, inactivation of the 5-HTT gene profoundly disturbs formation of the SSC with altered cytoarchitecture of cortical layer IV, the layer that contains synapses between thalamocortical terminals and their postsynaptic target neurons (Persico et al., 2001). Brains of 5-HTT KO mice display no or only very few barrels. Cell bodies as well as terminals, typically more dense in barrel septa, appear homogeneously distributed in layer IV of adult 5-HTT KO brains. Injections of a 5-HT synthesis inhibitor within a narrow time window of 2 days postnatally completely rescued formation of SSC barrel fields. Of note, heterozygous KO mice develop all SSC barrel fields, but frequently present irregularly shaped barrels and less defined cell gradients between septa and barrel hollows. These findings demonstrate that excessive concentrations of extracellular 5-HT are deleterious to SSC development and suggest that transient 5-HTT expression in thalamocortical neurons is responsible for barrel patterns in neonatal rodents, and its permissive action is required for normal barrel pattern formation, presumably by maintaining extracellular 5-HT concentrations below a critical threshold. Because normal synaptic density in SSC layer IV of 5-HTT KO mice was shown, it is more likely that 5-HT affects SSC cytoarchitecture by promoting dendritic growth toward the barrel hollows as well as by modulating cytokinetic movements of cortical granule cells, similar to concentration-dependent 5-HT modulation of cell migration described in other tissues. Since the gene/dose-dependent reduction in 5-HTT availability in heterozygous KO mice, which leads to a modest delay in 5-HT uptake but distinctive irregularities in barrel and septum shape, is similar to those reported in humans carrying low-activity allele of the 5-TT gene-linked polymorphic region (5-HTTLPR), it may be speculated that allelic variation in 5-HTT function also affects the human brain during development with due consequences for disease liability and therapeutic response (Persico et al., 2003).

These findings demonstrate that excessive amounts of extracellular 5-HT are detrimental to SSC development and suggest that transient 5-HTT expression and its permissive action is required for barrel pattern formation, presumably by maintaining extracellular 5-HT concentrations below a critical threshold. Two key players of serotonergic neurotransmission appear to mediate the deleterious effects of excess 5-HT: the 5-HTT and the 5-HT_{1B} receptor. Both molecules are expressed in primary sensory thalamic nuclei during the period when the segregation of thalamocortical projections occurs (Bennett-Clarke et al., 1996; Hansson et al., 1998). 5-HT is internalized via 5-HTT in thalamic neurons and is detectable in axon terminals (Cases et al., 1998; Lebrand et al., 1996). The presence of the VMAT2 within the same neurons allows internalized 5-HT to be stored in vesicles and used as a cotransmitter of glutamate. Lack of 5-HT degradation in MAOA KO mice as well as severe impairment of 5-HT clearance in mice with an inactivation of the 5-HTT results in an accumulation of 5-HT and overstimulation of 5-HT receptors all along thalamic neurons (Cases et al., 1998). Since 5-HT_{1B} receptors are known to inhibit the release of glutamate in the thalamocortical somatosensory pathway, excessive activation of 5-HT_{1B} receptors could prevent activity-dependent processes involved in the patterning of afferents and barrel structures. This hypothesis is supported by a recent study using a strategy of combined knockout of MAOA, 5-HTT, and 5-HT_{1B} receptor genes. While only partial disruption of the patterning of somatosensory thalamocortical projections was observed in 5-HTT KO, MAOA × 5-HTT double knockout (DKO) mice showed that 5-HT accumulation in the extracellular space causes total disruption of the patterning of these projections (Salichon et al., 2001). Moreover, the removal of 5-HT_{1B} receptors in MAOA and 5-HTT KO as well as in MAOA × 5-HTT DKO mice allows a normal segregation of the somatosensory projections as well as retinal axons in the lateral geniculate nucleus (Upton et al., 2002). These findings point to an essential role of the 5-HT_{1B} receptor in mediating the deleterious effects of excess 5-HT in the somatosensory system.

The effect of elevated extracellular 5-HT concentration on the modulation of programmed cell death during neural development was also investigated in early postnatal brains of 5-HTT KO mice. 5-HTT gene inactivation leads to a reduced number of apoptotic cells in striatum, thalamus, hypothalamus, cerebral cortex, and hippocampus on postnatal day 1 with differences displaying an increasing fronto-caudal gradient and regional specificity (Persico et al., 2003). These findings underscore the role of 5-HT in the regulation of programmed cell death during brain development, and suggest that pharmacological enhancement of serotoninergic neurotransmission may minimize pathological apoptosis.

The evidence that changes 5-HT system homeostasis exerts long-term effects on cortical development and adult brain plasticity may be an important step forward in establishing the psychobiological groundwork for a neurodevelopmental hypothesis of negative emotionality, aggressiveness, and violence (Lesch, 2003). Although there is converging evidence that serotonergic dysfunction contributes to anxiety-related behavior, the precise mechanism that renders 5-HTT-deficient mice more anxious and stress responsive remains to be elucidated. While increased 5-HT availability and activation of other serotonergic receptor subtypes that have been shown to mediate anxiety (e.g. 5-HT_{2C} receptor) may contribute to increased anxiety in rodent model, multiple downstream cellular pathways or neurocircuits, including noradrenergic, GABAergic, gluta-

matergic, and peptidergic transmission, as suggested by overexpression or targeted inactivation of critical genes within these systems, have been implicated to participate in the processing of this complex behavioral trait. Recent work has therefore been focused on a large number of genes that have known relevance in the neurocircuitries of fear and anxiety, although the knockout of some genes that appear not directly involved in anxiety may also lead to an anxiety-related phenotype.

3.4. Signal transduction and cellular pathways

As the next dimension of complexity, signaling through 5-HT receptors involves different transduction pathways, and each receptor subtype modulates distinct, though frequently interacting, second and third messenger systems and multiple effectors.

3.4.1. Adenylyl cyclase type VIII

Stress results in alterations in behavior and physiology that can be either adaptive or maladaptive. Although mice deficient in the Ca^{2+}-stimulated adenylyl cyclase type VIII (AC8) exhibit indices of anxiety comparable with that of wild-type mice at baseline, AC8 KO mice do not show normal increases in behavioral features of anxiety when subjected to repeated stress such as repetitive or post-restraint stress testing in the elevated plus maze test (Schaefer et al., 2000). Although these findings suggest a role for AC8 in the modulation of anxiety, the mechanism by which AC8 deficiency results in impaired stress-induced anxiety may be complex, involving impaired long-term depression in the CA1 region of the hippocampus and failure to activate CRE-binding protein (CREB) in the CA1 region after restraint stress. Interestingly, it was recently reported that CREB1 polymorphism predisposes to depressive disorder in a gender-specific manner further strengthening the assumption that this pathway is involved in emotion regulation (Zubenko et al., 2003).

3.4.2. Ca^{2+}-calmodulin kinase II

The gene of the effector enzyme Ca^{2+}-calmodulin kinase II (CaMKII), which participates in some intracellular responses to 5-HT receptor activation, has also been implicated in aggressive behavior by gene disruption (Chen et al., 1994). While CaMKII −/− mutants showed global behavioral impairment, male mice heterozygous for the inactivated CaMKII gene had a greater tendency to fight with each other when housed together. In detail, they showed enhanced offensive aggression, normal defensive aggression, and decreased fear-related responses.

3.4.3. G protein-activated inward rectifying potassium channel 2

The G protein-activated inward rectifying potassium (GIRK) channels regulate synaptic transmission and neuronal firing rates. The GIRK1–4 subunits exhibit unique but overlapping tissue localization patterns and contain cytoplasmic amino and carboxyl termini, two-transmembrane domains, and a hydrophobic pore region similar to other potassium-selective channels. Evidence for homo- and heteromultimerization of GIRK subunits has been derived from heterologous expression and biochemical studies (Wischmeyer et al., 1997). They are regulated by neurotransmitters and hormones through G protein-coupled receptors, including muscarinic M_2, dopamine D1–3, α_2-adrenoceptor, 5-HT_{1A}, adenosine A_1, $GABA_B$, μ, δ and κ-opioid, and somatostatin receptors. The GIRK2 (Kir 3.2) channel is abundantly expressed in the mammalian brain (Karschin et al., 1996) and co-localization of with dopamine receptors in the mesolimbic system and 5-HT_{1A} receptors in serotonergic raphe neurons suggests a role in modulation of motor activity and anxiety-like behavior (Luscher et al., 1997). Indeed, GIRK2-deficient mice show evidence for hyperactivity and reduced anxiety-like behavior with initially higher motor activity and slower habituation in a novel situation, increased levels of spontaneous locomotor activity during dark phase, and impaired habituation in the open field test (Blednov et al., 2001, 2002). After habituation, GIRK2 KO mice showed enhanced motor activity, which is modulated by D1 agonists and antagonists. Interestingly, increased expression and function of 5-HT_{1A} receptor-stimulated GIRK2 channels in mice with disruption of neural cell adhesion molecule (NCAM) gene may be causal for a lower excitability of target neurons for serotonergic fibers in the limbic system resulting in altered anxiety- and aggression-related behavior (Delling et al., 2002) (see also Section 3.5.3).

3.4.4. Neuronal nitric oxide synthase

The discovery of a considerable number of hyperaggressive mutant strains in the course of gene knockout experiments highlights the extraordinary diversity of genes involved in the genetic influence on emotionality. Interestingly, genetic support for a role of 5-HT in anxiety and aggression also derives from mice lacking specific genes, such as the neuronal nitric oxide synthase (nNOS), that either directly or indirectly affect 5-HT turnover or 5-HT receptor sensitivity. Male nNOS −/− mice and wild-type mice in which nNOS is pharmacologically suppressed are highly aggressive (Chiavegatto et al., 2001). Excessive aggressiveness and impulsiveness of nNOS KO mice depend on the presence of testosterone but seem to be caused by a selective decrease in 5-HT turnover and deficient 5-HT_{1A} and 5-HT_{1B} receptor function in brain regions regulating emotion. These findings indicate an interaction of nNOS and the 5-HT system mediated through 5-HT_{1A} and 5-HT_{1B} receptors, but the specific molecular mechanisms in anxiety and aggression remain to be clarified. Increased aggression and hypersexuality in male nNOS KO mice largely resemble the behavioral phenotype of the Brunner Syndrome caused by MAOA gene disruption (see also Section 3.2.1), suggesting common downstream pathways.

Interestingly, the impact of nNOS inactivation is gender-specific as female KO mice display reduced aggression during lactation (Gammie and Nelson, 1999). Whether this is also due to an interaction with the serotonergic system remains to be investigated.

3.5. Developmental specification serotonergic neurons: setting the stage

Despite the widespread importance of the central serotonergic neurotransmitter system, little is known about the molecular mechanisms controlling the development of 5-HT neurons. Several regulatory genes including transcription factors, other morphogenetically relevant regulators of gene expression, neurotrophins and growth factors as well as 5-HT itself contribute to the specification, differentiation, and phenotype maintenance of the raphe serotonergic system (Fig. 1).

Induction of the floor plate at the ventral midline of the neural tube is one of the earliest events in the establishment of dorsoventral polarity in the vertebrate brain. Fibroblast growth factors (Fgf4 and 8) and the secreted signaling molecule Sonic hedgehog (Shh) signals control serotonergic (and dopaminergic) cell fate in the anterior neural plate (Hynes et al., 2000; Ye et al., 1998). Generation of 5-HT neurons in the neural tube depends on the activity of the notochord and floor plate-derived Shh. Fgf4 and Fgf8 expressed in the primitive streak and isthmus region, respectively, have been suggested to participate in the formation of an induction center that specifies the identity and location of rostral 5-HT neurons. Shh together with unidentified secreted signaling molecules, but not Fgf8, induce the generation of the caudal cluster of 5-HT neurons.

In addition, several transcription factors participate in the development of 5-HT neurons. The homeobox gene Nkx2.2 and the zinc-finger transcription factor Gli2 are two downstream targets of Shh that operate during early stages of neurogenesis in the hindbrain and midbrain. Knockout of Nkx2.2 results in the absence of some serotonergic neurons in rhombomere 2 of the hindbrain (Briscoe et al., 1999), whereas elimination of Gli2 results in a partial loss and abnormal location of remaining 5-HT neurons in the ventral midline (Matise et al., 1998). Even within the relatively circumscribed serotonergic raphe complex, gene expression in discrete subsystems appears to be differentially controlled by transcriptional regulators. The transcription factor Gata3 is expressed broadly during embryogenesis, including in many but not all 5-HT raphe neurons (van Doorninck et al., 1999). Gata3 seems to play a critical role in the development of serotonergic neurons of the caudal raphe nuclei and thus in locomotor performance (Matise et al., 1998).

Based on the increasing body of evidence that genetically driven variability of transcriptional regulators, neurotrophins, and growth factors, is associated with behavioral traits, research is giving more and more emphasis to the investigations of the molecular basis of gene regulation in complex behavior. Despite their remarkable impact on brain development and behavioral functions, mechanisms underlying the developmental genetics of serotonergic neurons are only beginning to emerge. Nevertheless, unraveling the interactions of these determinants of development remains a daunting task.

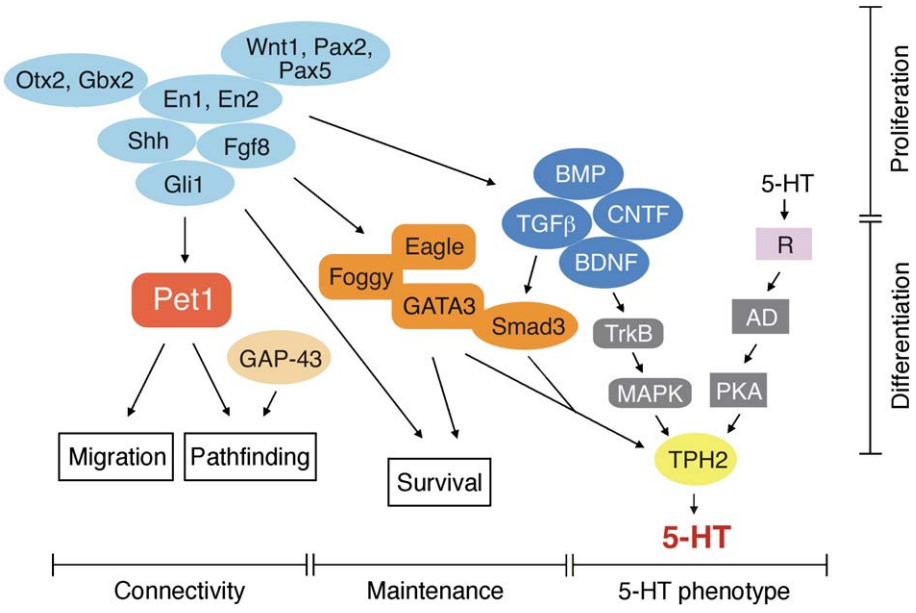

Fig. 1. Genetic pathways in the development of the raphe serotonin (5-HT) system (modified from Lesch, 2001b). Wnt1, Pax2, Pax5, Otx2, Gbx2, En1, En2, Shh, Fgf8, Gli1, Pet1, Foggy, Eagle, and GATA3 are transcription factors. GAP-43, Smad3, BMP, TGFβ, CNTF, and BDNF are neurotrophins and other growth factors. TrkB, neurotrophin receptor; MAPK, MAP kinase; AD, adenylyl cyclase; PKA, protein kinase A; TPH2, tryptophan hydroxylase 2; R, receptor.

3.5.1. Transcription factor Pet1

Although many transcription factors have been implicated in the development of 5-HT neurons, they are also important for the development of many other neuronal and nonneuronal cell types. While Nkx2.2 and Gli2 are also required for induction of floor plate and adjacent cells including both serotonergic and dopaminergic neurons throughout the midbrain, hindbrain and spinal cord (van Doorninck et al., 1999), expression of Pet1, an ETS domain transcription factor, is restricted to the rostrocaudal extent of hindbrain raphe nuclei and closely associated with developing serotonergic neurons in the raphe nuclei (Hendricks et al., 1999) (Fig. 1). Pet1 is therefore likely to be distinct from other factors because its expression pattern suggests that it performs a strictly serotonergic-specific function in the brain. Moreover, consensus Pet1-binding motifs are present in the transcriptional regulatory regions of both the human and murine 5-HT_{1A} receptor, 5-HTT, tryptophan hydroxylases (TPH1 and 2), and aromatic L-amino acid decarboxylase (Aaad) genes whose expression profile is characteristic of the serotonergic neuron phenotype, i.e. 5-HT synthesis, release, uptake, and metabolism (Table 1). In the rat dorsal and median raphe, 5-HT neurons begin to appear at approximately embryonic day 11 (E11) and peaking at E13–E14. In these nuclei, it is thought that serotonergic neuron precursors begin to produce 5-HT near the time of their last cell division. The detection of Pet1 as early as E12.5 in the rostral cluster suggests that it is expressed in 5-HT neuron precursors during their terminal differentiation, consistent with its expression before the appearance of 5-HT. Taken together, these findings identify Pet1 as a critical regulator of serotonergic system specification.

While nearly all serotonergic neurons fail to differentiate in mice lacking Pet1, the remaining exhibit deficient expression of genes required for 5-HT synthesis, uptake, and vesicular storage (Hendricks et al., 2003). In target fields including cortex and hippocampus, 5-HT specific fibers as well as 5-HT and 5-HIAA concentrations were also dramatically reduced in Pet1 KO mice, whereas no major cytoarchitectural abnormalities in nuclear groups of several brain regions were detected. Interestingly, Pet1-deficient mice show evidence for increased anxiety-like behavior in the elevated plus maze test and enhanced aggressiveness in the Resident–Intruder test as a consequence of disrupted 5-HT system development. These findings further support the notion that Pet1 is a critical determinant of serotonergic neuron identity. Moreover, the Pet1-dependent transcriptional program appears to couple 5-HT neuron differentiation during brain development to serotonergic modulation of behavior related to anxiety and aggression in adulthood.

Beyond the point of transcription initiation and neurotrophin action, the role of messenger RNA elongation and other mechanisms of neural gene regulation are increasingly attracting systematic scrutiny. Foggy, a phosphorylation-dependent, dual regulator of transcript elongation affects development of 5-HT (and dopamine)-containing neurons in the zebrafish (Guo et al., 2000). In the fruit fly, Eagle, a zinc finger transcription factor with homology to the steroid receptor family is required for the specification of 5-HT

Table 1
Mice with inactivation of serotonergic genes displaying an anxiety-like or related behavioral phenotype

Knockout	Effect of anxiety-related behavior	Additional behavioral phenotypes, special features	Authors
Serotonergic neuron phenotype-specific genes			
5-HT_{1A} receptor	↑	Consistent in different genetic backgrounds	Heisler et al., 1998 Ramboz et al., 1998 Parks et al., 1998 Sibille et al., 2000
5-HT_{1B} receptor	↓	Aggression ↑	Brunner et al., 1999
5-HT transporter	↑	Stress reactivity ↑ Aggression ↓	Holmes et al., 2003 Holmes et al., 2002
TPH1	–	normal 5-HT in brain	Walther et al., 2003
TPH2	n.d.		
MAOA	↑ (?)	aggression ↑ stress reactivity ↑	Cases et al., 1995 Seif and De Maeyer, 1999
Signal transduction			
AC VIII	↑		Schaefer et al., 2000
CamKII	↓	offensive aggression ↑	Chen et al., 1994
GIRK2	↓	hyperactivity	Blednov et al., 2001
nNOS	↓ ?	impulsivity and aggression ↑	Chiavegatto et al., 2001
Developmental factors			
NCAM	↑	5-HT_{1A} response ↑ GIRK2 ↑	Delling et al., 2002 Stork et al., 1999
Pet1	↑	aggression ↑	Hendricks et al., 2003
BDNF	↑*	*conditional knockout constitutive knockout: +/–, aggression ↑ –/–, not viable	Rios et al., 2001 Lyons et al., 1999
Other 5-HT-related systems			
tPA	↑	GAP-43, a growth-associated protein of growing 5-HT axons ↓	Pawlak et al., 2003
Neurokinin 1 receptor	↑	serotonergic function ↑	Santarelli et al., 2001

MAOA, monoamine oxidase A; AC VIII, adenylyl cyclase type VIII; CamKII, Ca^{2+}-calmodulin kinase II; GIRK2, G protein-activated inward rectifying potassium 2; nNOS, neuronal nitric oxide synthase, NCAM, neural cell adhesion molecule; Pet1, ETS domain transcription factor; BDNF, brain-derived neurotrophic factor; tPA, serine protease tissue-plasminogen activator (tPA); ↑/↓, increase/decrease in anxiety-related behavior; –, no effect.
n.d., not determined.

neurons (Lundell and Hirsh, 1998). The human orthologs of Foggy and Eagle remain to be identified and their roles in anxiety to be determined.

3.5.2. Brain-derived neurotrophic factor

Several other neurotrophins and growth factors also modulate the phenotype of serotonergic neurons. These include family members of the neurotrophins, such as transforming growth factor-β (TGF-β), bone morphogenetic protein (BMP), and neurokines (ciliary neurotrophic factor, CNTF) (Galter and Unsicker, 2000a,b). 5-HT itself regulates the serotonergic phenotype of neurons by sequential activation of the 5-HT_{1A} receptor, brain-derived neurotrophic factor (BDNF), and its receptor trkB as well as a wide spectrum of signal transduction pathways. In particular, transcriptional regulation appears to be dependent on stimulation of the adenylyl cyclase/protein kinase A signaling pathway mediated by a family of cyclic AMP-responsive nuclear factors, including CREB, CREM, and ATF-1 (Herdegen and Leah, 1998). These factors contain the basic domain/leucine zipper motifs and bind as dimers to cAMP-responsive elements (CREs). Galter and Unsicker (2000a,b) have therefore proposed the neurotrophin receptor trkB as the master control protein that integrates a diverse array of signals that elicit and maintain serotonergic differentiation.

BDNF is involved in a variety of trophic and modulatory effects that include a critical role in the development and plasticity of dopaminergic, serotonergic, and other neurons (Bonhoeffer, 1996; Schuman, 1999). Specifically, BDNF enhances differentiation of 5-HT neurons during embryonic development and prevents neurotoxin-induced serotonergic denervation in adult brain (Frechilla et al., 2000; Galter and Unsicker, 2000a,b). Furthermore, human fetal mesencephalic cultured cells treated with BDNF exhibit greater neuronal survival and increased tissue 5-HT concentrations (Spenger et al., 1995). BDNF treatment of E14 rat embryos induced a 2-fold increase in the number of raphe 5-HT neurons and produced a marked extension and ramification of their neurites with greater expression of 5-HTT, 5-HT_{1A}, and 5-HT_{1B} receptors (Galter and Unsicker, 2000a; Zhou and Iacovitti, 2000). Reduced expression of BDNF modifies synaptic plasticity resulting in specific alterations in spatial learning and memory processes, emotionality, and motor activity in KO mice (Carter et al., 2002; Kernie et al., 2000; Minichiello et al., 1999), whereas targeted inactivation of the BDNF receptor, TrkB, leads to neuronal loss and cortical degenerative changes (Vitalis et al., 2002). In addition, BDNF mediates the effects of repeated stress exposure and long-term antidepressant treatment on neurogenesis and neuronal survival in the hippocampus (D'Sa and Duman, 2002). These findings converge with reduced hippocampal plasticity reflected by a reduced hippocampal volume and hippocampus-related memory deficiency plays a critical role in the pathophysiology of emotional and stress-related disorders (Duman, 2002).

Mice completely lacking BDNF have reduced sensory neuron survival, other neuronal deficits, and are viable only a few weeks (Ernfors et al., 1995). Heterozygote BDNF +/− mice exhibit gene/dose-dependent reductions in BDNF expression in forebrain, hippocampus, and some hypothalamic nuclei (Kernie et al., 2000; MacQueen et al., 2001) as well as decreased striatal dopamine content, decreased potassium-elicited dopamine release (Dluzen et al., 2002), and some evidence for decreased concentrations of forebrain 5-HT concentrations and fiber densities at 18 months of age (Lyons et al., 1999). Furthermore, learning deficits and hyperactivity was revealed in BDNF +/− mice (Kernie et al., 2000). They also develop intermale aggressiveness in the resident–intruder test (Lyons et al., 1999), but do not show increased anxiety in the elevated plus maze, nor differences in the antidepressant-sensitive forced swim test (MacQueen et al., 2001). However, conditional deletion of the BDNF gene in the postnatal brain leads to increased anxiety-like behavior in the light–dark box, deficits in context-dependent learning in a fear conditioning paradigm, and hyperactivity (Rios et al., 2001). Both conditional and constitutive BDNF KO mice also exhibit obesity, with hyperphagia, elevated serum glucose, insulin and leptin levels, and elevated cell fat content (Kernie et al., 2000; Rios et al., 2001).

Possible gene-interactive alterations in 5-HT function and BDNF expression was recently evaluated in mice with a combined manipulation of the genes for the 5-HTT and BDNF. Interestingly, male but not female 5-HTT −/− × BDNF+/− DKO mice showed further decreases in brain 5-HT concentrations as well as further increases in anxiety-like behavior and stress reactivity compared to 5-HTT −/− × BDNF+/+ controls (Murphy, personal communication). These findings support the notion of critical role of gene–gene interaction in brain plasticity related to anxiety and related disorders.

3.5.3. Neural cell adhesion molecule

The neural cell adhesion molecule (NCAM) plays a critical role during brain development and in adult plasticity. In particular, NCAM is involved in neuronal migration, neurite outgrowth, synaptic plasticity, and emotional behavior (Schachner, 1997). NCAM-deficient mice display elevated both anxiety and aggression levels (Stork et al., 1999). Although 5-HT_{1A} binding as well as brain 5-HT and 5-HIAA tissue concentrations were unaltered, lower doses of 5-HT_{1A} agonists are necessary to reduce anxiety and aggressiveness in the NCAM −/− mice, suggesting a functional change in the 5-HT_{1A} receptor (Stork et al., 1999). Interestingly, the expression of one of the effectors of the 5-HT_{1A} receptor, the G protein-activated inward rectifying potassium channel 2 (GIRK2) is greatly upregulated in NCAM KO mice, thus identifying disrupted 5-HT_{1A}-activated cellular pathways as an additional cause for their anxiety- and aggression-related behavior (Delling et al., 2002) (see also Section 3.4.3). Taken together, these findings indicate an involvement of NCAM impacting on 5-

HT system function through the 5-HT_{1A} receptors and its effector, but the specific molecular mechanisms in emotional behavior remain to be elucidated in more detail.

3.6. Other 5-HT-related systems

3.6.1. Tissue-plasminogen activator and growth-associated protein-43

Adaptive responses to stressful events comprise physiological processes and behavior aimed at sustaining homeostasis, while severe stress may modify this response and lead to exaggerated fear reaction and persisting anxiety and depression. At the center of the functional neuroanatomy of the stress circuit are the amygdala and the hippocampus, which both exhibit dendritic remodeling following repeated inescapable stress. Although several key players of the stress circuit have been characterized, the mechanism that underlies stress-induced neural plasticity leading to anxiety and associated cognitive impairment remains to be elucidated. Recently, Pawlak et al. (2003) identified acute restraint stress-induced upregulation of the serine protease tissue-plasminogen activator (tPA) in the amygdala as a critical mechanism in stress-related neural remodeling which is either adaptive and directed toward attenuation of the deleterious impact of stress on the brain or is reflecting the interference with protective mechanisms. Targeted disruption of the tPA gene in mice resulted in attenuated anxiety-like behavior and maladaptive endocrine response as well as compromised neural plasticity. These findings suggest that tPA represents a signal to the postsynaptic machinery to phosphorylate extracellular signal-regulated kinase 1/2 (ERK1/2), a trigger for postsynaptic plasticity-related events. Furthermore, axonal remodeling reflected by decreases in expression of growth-associated protein-43 (GAP-43), a morphogenic protein expressed in growing 5-HT axons and thus a marker of presynaptic plasticity, in the amygdala but not in hippocampus. Interestingly, a gene/dose-dependent failure of 5-HT axons to innervate selected forebrain regions including the somatosensory cortex and hippocampus but not the amygdala was revealed in GAP-43 KO mice suggesting GAP-43 as a key regulator in normal path finding and arborization of 5-HT axons during early brain development (Donovan et al., 2002; Maier et al., 1999). However, it remains to be clarified whether GAP-43 participates also in adult plasticity of the hippocampus or amygdala, and whether GAP-43 KO mice exhibit changes in anxiety-related behavior.

3.6.2. Neurokinin 1 receptor

Although substance P and its receptor, neurokinin 1 receptor (NK1R), have been implicated in the control of mood, anxiety, and stress, the efficacy of NK1R antagonists as both antidepressants and anxiolytics has been a matter of considerable debate (Lesch, 2001a). Santarelli et al. (2001) have recently made a strong argument for a critical role of the SP/NK1R system the modulation of anxiety-related behaviors in mice. Targeted inactivation of the NK1R produced a phenotype that is associated with an increase of fear and anxiety in the elevated plus maze, novelty suppressed feeding, and maternal separation paradigms. Results derived from pharmacologic, immunohistochemical, autoradiographic, endocrine, and electrophysiological studies convincingly identify the 5-HT system as a important participant in anxiety-related responses to NK1R KO, while an association of the NK1R with noradrenergic neurons seems to mediate this behavioral phenotype. Thus, NK1R antagonists may exert their anxiolytic effect by modulating the activity of noradrenergic neurons, which in turn modulate serotonergic function.

4. Clinical implications and outlook

Mutant mice of genes controlling 5-HT system development and plasticity, of genes establishing 5-HT neuron identity, and of genes modulating 5-HT receptor-mediated signal transduction and cellular pathways provide practical models to study how genomic variation in these genes modulates human emotional behavior (Lesch, 2001a). Allelic variation in the expression of human genes and function of their respective protein products, which are determinants of the serotonergic neuron phenotype, has been implicated in anxiety-related traits, such as 5-HT_{1A}, and 5-HTT (Reif and Lesch, 2003), and aggressive behavior, such as 5-HT_{1B}, and MAOA (Lappalainen et al., 1998; Lesch and Merschdorf, 2000; Samochowiec et al., 1999) (Table 2). For instance, consistent with the finding that 5-HTT gene

Table 2
Functional serotonergic gene variations associated with behavioral phenotype, psychopathology and psychiatric disorders

Gene	Functional Variation	Behavioral traits	Psychopathology/ disorders
5-HT receptors			
1A	C-1019G	Anxiety, depression	Depression, suicidality
1B	G861C (linkage disequilibrium with promoter haplotype)	Impulsivity, aggression	Alcoholism
2C	Cys23Ser HTR2C-LPR	Feeding, learning and memory, anxiety?	Hallucinatory psychosis, eating disorders
5-HTT	5HTT-LPR	Anxiety, depression stress reactivity, aggression	Depression/ suicidality, alcoholism, OCD, autism, ADHD, eating disorders
MAOA	MAOA-LPR	Aggression, anxiety antisocial behavior	Alcoholism, panic disorder, antisocial personality disorder

inactivation in mice leads to increased anxiety-like and reduced aggressive behavior, the allelic variation of human 5-HTT polymorphism is associated with increased trait anxiety/stress reactivity and neural responses to fear (Hariri et al., 2002; Lesch, 2003; Lesch et al., 1996). Moreover, genetically driven variation in 5-HTT function also modifies the risk for anxiety and depressive disorders and their response to treatment (Caspi et al., 2003; Collier et al., 1996; Lesch, 2001b; Lesch and Mössner, 1998). Another example is a repeat polymorphism in the promoter region of the human and nonhuman primate MAOA gene that differentially modulates gene transcription (Bennett et al., submitted for publication; Deckert et al., 1999). Allelic variation in MAOA gene expression and enzyme activity is not only associated with aggressiveness and violence but also with panic disorder and antisocial personality disorder in a gender-specific fashion (Deckert et al., 1999; Samochowiec et al., 1999).

Most notably, genetic influences are not the only pathway that lead to individual differences in personality dimensions, behavior, and psychopathology. Complex traits like anxiety, depression, or aggression are most likely to be generated by a complex interaction of environmental and experiential factors with a number of genes and their products as documented extensively for the 5-HTT (Barr et al., 2003; Bennett et al., 2002; Caspi et al., 2003; Champoux et al., 2002; Lesch et al., 2002). Even pivotal regulatory proteins of neurocircuits have only a modest impact, while noise from epigenetic mechanisms obstructs identification of relevant gene variants. Although current methods for the detection of gene–environment interaction in behavioral genetics are largely indirect, the most pertinent consequence of gene identification for behavioral traits may be that it will provide the tools required to systematically elucidate the effects of gene–environment interaction.

Finally, future benefits will stem from the potential development of strategies involving spatio-temporally specific conditional knockouts with and gene transfer technology that could facilitate novel drug design (Lesch, 2001a). Paralleling the resolution of gene–gene and gene–environment interactions and the fading dogma that neurons are highly vulnerable and their capacity for regeneration, reproducibility, and plasticity is limited, it is being realized that advanced gene transfer strategies may eventually be applicable to complex behavioral disorders.

References

Azmitia, E.C., Whitaker-Azmitia, P.M., 1997. Development and adult plasticity of serotonergic neurons and their target cells. In: Baumgarten, H.G., Göthert, M. (Eds.), Serotonergic Neurons and 5-HT Receptors in the CNS, vol. 129. Springer, Berlin, pp. 1–39.

Barnes, N.M., Sharp, T., 1999. A review of central 5-HT receptors and their function. Neuropharmacology 38, 1083–1152.

Barr, C.S., Newman, T.K., Becker, M.L., Champoux, M., Lesch, K.P., Suomi, S.J., Goldman, D., Higley, J.D., 2003. Serotonin transporter gene variation is associated with alcohol sensitivity in rhesus macaques exposed to early-life stress. Alcohol., Clin. Exp. Res. 27, 812–817.

Bellivier, F., Leboyer, M., Courtet, P., Buresi, C., Beaufils, B., Samolyk, D., Allilaire, J.F., Feingold, J., Mallet, J., Malafosse, A., 1998. Association between the tryptophan hydroxylase gene and manic-depressive illness [see comments]. Arch. Gen. Psychiatry 55, 33–37.

Bengel, D., Murphy, D.L., Andrews, A.M., Wichems, C.H., Feltner, D., Heils, A., Mossner, R., Westphal, H., Lesch, K.P., 1998. Altered brain serotonin homeostasis and locomotor insensitivity to 3,4-methylenedioxymethamphetamine (“Ecstasy”) in serotonin transporter-deficient mice. Mol. Pharmacol. 53, 649–655.

Bennett, A.J., Lesch, K.P., Heils, A., Long, J.C., Lorenz, J.G., Shoaf, S.E., Champoux, M., Suomi, S.J., Linnoila, M.V., Higley, J.D., 2002. Early experience and serotonin transporter gene variation interact to influence primate CNS function. Mol. Psychiatry 7, 118–122.

Bennett, A.J., Syagailo, Y., Higley, J.D., Champoux, M., Graessle, M., Suomi, S.J., Lesch, K.P., 2003. MAOA genotype interacts with early adverse experience to influence aggression and injuries in primates, submitted for publication.

Bennett-Clarke, C.A., Chiaia, N.L., Rhoades, R.W., 1996. Thalamocortical afferents in rat transiently express high-affinity serotonin uptake sites. Brain Res. 733, 301–306.

Berlin, I., Warot, D., Legout, V., Guillemant, S., Schollnhammer, G., Puech, A.J., 1998. Blunted 5-HT1A-receptor agonist-induced corticotropin and cortisol responses after long-term ipsapirone and fluoxetine administration to healthy subjects. Clin. Pharmacol. Ther. 63, 428–436.

Blednov, Y.A., Stoffel, M., Chang, S.R., Harris, R.A., 2001. GIRK2 deficient mice. Evidence for hyperactivity and reduced anxiety. Physiol. Behav. 74, 109–117.

Blednov, Y.A., Stoffel, M., Cooper, R., Wallace, D., Mane, N., Harris, R.A., 2002. Hyperactivity and dopamine D1 receptor activation in mice lacking girk2 channels. Psychopharmacology (Berl.) 159, 370–378.

Blier, P., de Montigny, C., 1998. Possible serotonergic mechanisms underlying the antidepressant and anti-obsessive–compulsive disorder responses. Biol. Psychiatry 44, 313–323.

Bonasera, S.J., Tecott, L.H., 2000. Mouse models of serotonin receptor function: toward a genetic dissection of serotonin systems. Pharmacol. Ther. 88, 133–142.

Bonhoeffer, T., 1996. Neurotrophins and activity-dependent development of the neocortex. Curr. Opin. Neurobiol. 6, 119–126.

Boularand, S., Darmon, M.C., Mallet, J., 1995. The human tryptophan hydroxylase gene. An unusual splicing complexity in the 5′ -untranslated region. J. Biol. Chem. 270, 3748–3756.

Briscoe, J., Sussel, L., Serup, P., Hartigan-O’Connor, D., Jessell, T.M., Rubenstein, J.L., Ericson, J., 1999. Homeobox gene Nkx2.2 and specification of neuronal identity by graded Sonic hedgehog signalling. Nature 398, 622–627.

Brunner, H.G., Nelen, M., Breakefield, X.O., Ropers, H.H., van Oost, B.A., 1993. Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. Science 262, 578–580.

Brunner, D., Buhot, M.C., Hen, R., Hofer, M., 1999. Anxiety, motor activation, and maternal–infant interactions in 5HT1B knockout mice. Behav. Neurosci. 113, 587–601.

Burnet, H., Bevengut, M., Chakri, F., Bou-Flores, C., Coulon, P., Gaytan, S., Pasaro, R., Hilaire, G., 2001. Altered respiratory activity and respiratory regulations in adult monoamine oxidase A-deficient mice. J. Neurosci. 21, 5212–5221.

Carter, A.R., Chen, C., Schwartz, P.M., Segal, R.A., 2002. Brain-derived neurotrophic factor modulates cerebellar plasticity and synaptic ultrastructure. J. Neurosci. 22, 1316–1327.

Cases, O., Seif, I., Grimsby, J., Gaspar, P., Chen, K., Pournin, S., Muller, U., Aguet, M., Babinet, C., Shih, J.C., et al., 1995. Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA [see comments]. Science 268, 1763–1766.

Cases, O., Lebrand, C., Giros, B., Vitalis, T., De Maeyer, E., Caron, M.G., Price, D.J., Gaspar, P., Seif, I., 1998. Plasma membrane transporters of

serotonin, dopamine, and norepinephrine mediate serotonin accumulation in atypical locations in the developing brain of monoamine oxidase A knock-outs. J. Neurosci. 18, 6914–6927.

Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A., Poulton, R., 2003. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301, 386–389.

Champoux, M., Bennett, A., Shannon, C., Higley, J.D., Lesch, K.P., Suomi, S.J., 2002. Serotonin transporter gene polymorphism, differential early rearing, and behavior in rhesus monkey neonates. Mol. Psychiatry 7, 1058–1063.

Cheetham, S.C., Crompton, M.R., Katona, C.L., Horton, R.W., 1990. Brain 5-HT1 binding sites in depressed suicides. Psychopharmacology (Berl.) 102, 544–548.

Chen, C., Rainnie, D.G., Greene, R.W., Tonegawa, S., 1994. Abnormal fear response and aggressive behavior in mutant mice deficient for alpha-calcium-calmodulin kinase II. Science 266, 291–294.

Chen, J.J., Li, Z., Pan, H., Murphy, D.L., Tamir, H., Koepsell, H., Gershon, M.D., 2001. Maintenance of serotonin in the intestinal mucosa and ganglia of mice that lack the high-affinity serotonin transporter: abnormal intestinal motility and the expression of cation transporters. J. Neurosci. 21, 6348–6361.

Chiavegatto, S., Dawson, V.L., Mamounas, L.A., Koliatsos, V.E., Dawson, T.M., Nelson, R.J., 2001. Brain serotonin dysfunction accounts for aggression in male mice lacking neuronal nitric oxide synthase. Proc. Natl. Acad. Sci. U. S. A. 98, 1277–1281.

Collier, D.A., Stober, G., Li, T., Heils, A., Catalano, M., Di Bella, D., Arranz, M.J., Murray, R.M., Vallada, H.P., Bengel, D., Muller, C.R., Roberts, G.W., Smeraldi, E., Kirov, G., Sham, P., Lesch, K.P., 1996. A novel functional polymorphism within the promoter of the serotonin transporter gene: possible role in susceptibility to affective disorders. Mol. Psychiatry 1, 453–460.

Crawley, J.N., 1999. Behavioral phenotyping of transgenic and knockout mice: experimental design and evaluation of general health, sensory functions, motor abilities, and specific behavioral tests. Brain Res. 835, 18–26.

Crawley, J.N., Paylor, R., 1997. A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. Horm. Behav. 31, 197–211.

Deckert, J., Catalano, M., Syagailo, Y.V., Bosi, M., Okladnova, O., Di Bella, D., Nothen, M.M., Maffei, P., Franke, P., Fritze, J., Maier, W., Propping, P., Beckmann, H., Bellodi, L., Lesch, K.P., 1999. Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder. Hum. Mol. Genet. 8, 621–624.

Delling, M., Wischmeyer, E., Dityatev, A., Sytnyk, V., Veh, R.W., Karschin, A., Schachner, M., 2002. The neural cell adhesion molecule regulates cell-surface delivery of G-protein-activated inwardly rectifying potassium channels via lipid rafts. J. Neurosci. 22, 7154–7164.

De Vry, J., 1995. 5-HT1A receptor agonists: recent developments and controversial issues. Psychopharmacology (Berl.) 121, 1–26.

Di Pino, G., Mössner, R., Lesch, K.P., Lauder, J.M., Persico, A.M., 2003. Serotonin roles in neurodevelopment: more than just neural transmission. Curr. Neuropharmacol. (in press).

Dluzen, D.E., Anderson, L.I., McDermott, J.L., Kucera, J., Walro, J.M., 2002. Striatal dopamine output is compromised within +/− BDNF mice. Synapse 43, 112–117.

Donovan, S.L., Mamounas, L.A., Andrews, A.M., Blue, M.E., McCasland, J.S., 2002. GAP-43 is critical for normal development of the serotonergic innervation in forebrain. J. Neurosci. 22, 3543–3552.

Drevets, W.C., Frank, E., Price, J.C., Kupfer, D.J., Holt, D., Greer, P.J., Huang, Y., Gautier, C., Mathis, C., 1999. PET imaging of serotonin 1A receptor binding in depression. Biol. Psychiatry 46, 1375–1387.

D'Sa, C., Duman, R.S., 2002. Antidepressants and neuroplasticity. Bipolar Disord. 4, 183–194.

Duman, R.S., 2002. Synaptic plasticity and mood disorders. Mol. Psychiatry 7 (Suppl. 1), S29–S34.

Eley, T.C., Plomin, R., 1997. Genetic analyses of emotionality. Curr. Opin. Neurobiol. 7, 279–284.

Ernfors, P., Kucera, J., Lee, K.F., Loring, J., Jaenisch, R., 1995. Studies on the physiological role of brain-derived neurotrophic factor and neurotrophin-3 in knockout mice. Int. J. Dev. Biol. 39, 799–807.

Fabre, V., Rioux, A., Lesch, K.P., Murphy, D.L., Lanfumey, L., Hamon, M., Marres, M.P., 1999. Altered expression and coupling of the serotonin 5-HT1A and 5-HT1B receptors in knock-out mice lacking the 5-HT transporter. Eur. J. Neurosci. 12, 2299–2310.

Finn, D.A., Rutledge-Gorman, M.T., Crabbe, J.C., 2003. Genetic animal models of anxiety. Neurogenetics 4, 109–135.

Flint, J., Corley, R., 1996. Do animal models have a place in the genetic analysis of quantitative human behavioural traits? J. Mol. Med. 74, 515–521.

Flint, J., Corley, R., DeFries, J.C., Fulker, D.W., Gray, J.A., Miller, S., Collins, A.C., 1995. A simple genetic basis for a complex psychological trait in laboratory mice. Science 269, 1432–1435.

Flugge, G., 1995. Dynamics of central nervous 5-HT1A-receptors under psychosocial stress. J. Neurosci. 15, 7132–7140.

Francis, D., Diorio, J., Liu, D., Meaney, M.J., 1999. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. Science 286, 1155–1158.

Francis, D.D., Szegda, K., Campbell, G., Martin, W.D., Insel, T.R., 2003. Epigenetic sources of behavioral differences in mice. Nat. Neurosci. 6, 445–446.

Frechilla, D., Insausti, R., Ruiz-Golvano, P., Garcia-Osta, A., Rubio, M.P., Almendral, J.M., Del Rio, J., 2000. Implanted BDNF-producing fibroblasts prevent neurotoxin-induced serotonergic denervation in the rat striatum. Brain Res. Mol. Brain Res. 76, 306–314.

Furlong, R.A., Ho, L., Rubinsztein, J.S., Walsh, C., Paykel, E.S., Rubinsztein, D.C., 1998. No association of the tryptophan hydroxylase gene with bipolar affective disorder, unipolar affective disorder, or suicidal behaviour in major affective disorder. Am. J. Med. Genet. 81, 245–247.

Galter, D., Unsicker, K., 2000a. Brain-derived neurotrophic factor and trkB are essential for cAMP-mediated induction of the serotonergic neuronal phenotype. J. Neurosci. Res. 61, 295–301.

Galter, D., Unsicker, K., 2000b. Sequential activation of the 5-HT1(A) serotonin receptor and TrkB induces the serotonergic neuronal phenotype. Mol. Cell. Neurosci. 15, 446–455.

Gammie, S.C., Nelson, R.J., 1999. Maternal aggression is reduced in neuronal nitric oxide synthase-deficient mice. J. Neurosci. 19, 8027–8035.

Gershenfeld, H.K., Paul, S.M., 1997. Mapping quantitative trait loci for fear-like behaviors in mice. Genomics 46, 1–8.

Gobbi, G., Murphy, D.L., Lesch, K., Blier, P., 2001. Modifications of the serotonergic system in mice lacking serotonin transporters: an in vivo electrophysiological study. J. Pharmacol. Exp. Ther. 296, 987–995.

Gorman, J.M., Kent, J.M., Sullivan, G.M., Coplan, J.D., 2000. Neuroanatomical hypothesis of panic disorder, revised. Am. J. Psychiatry 157, 493–505.

Grailhe, R., Waeber, C., Dulawa, S.C., Hornung, J.P., Zhuang, X., Brunner, D., Geyer, M.A., Hen, R., 1999. Increased exploratory activity and altered response to LSD in mice lacking the 5-HT(5A) receptor. Neuron 22, 581–591.

Griebel, G., 1995. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmacol. Ther. 65, 319–395.

Griebel, G., Belzung, C., Perrault, G., Sanger, D.J., 2000. Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. Psychopharmacology (Berl.) 148, 164–170.

Groenink, L., Pattij, T., De Jongh, R., Van der Gugten, J., Oosting, R.S., Dirks, A., Olivier, B., 2003. 5-HT(1A) receptor knockout mice and mice overexpressing corticotropin-releasing hormone in models of anxiety. Eur. J. Pharmacol. 463, 185–197.

Gross, C., Zhuang, X., Stark, K., Ramboz, S., Oosting, R., Kirby, L., Santarelli, L., Beck, S., Hen, R., 2002. Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. Nature 416, 396–400.

Guo, S., Yamaguchi, Y., Schilbach, S., Wada, T., Lee, J., Goddard, A., French, D., Handa, H., Rosenthal, A., 2000. A regulator of transcriptional elongation controls vertebrate neuronal development. Nature 408, 366–369.

Hamon, M., Gozlan, H., El Mestikawy, S., Emerit, M.B., Boanos, F., Schlechter, L., 1990. The central 5-HT1A receptors: pharmacological, biochemical, functional and regulatory properties. Ann. N.Y. Acad. Sci. 600, 114–131.

Hansson, S.R., Mezey, E., Hoffman, B.J., 1998. Serotonin transporter messenger RNA in the developing rat brain: early expression in serotonergic neurons and transient expression in non-serotonergic neurons. Neuroscience 83, 1185–1201.

Hariri, A.R., Mattay, V.S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., Egan, M.F., Weinberger, D.R., 2002. Serotonin transporter genetic variation and the response of the human amygdala. Science 297, 400–403.

Hedlund, P.B., Danielson, P.E., Thomas, E.A., Slanina, K., Carson, M.J., Sutcliffe, J.G., 2003. No hypothermic response to serotonin in 5-HT7 receptor knockout mice. Proc. Natl. Acad. Sci. U. S. A. 100, 1375–1380.

Heisler, L.K., Tecott, L.H., 1999. Knockout corner—neurobehavioural consequences of a serotonin 5-HT(2C) receptor gene mutation. Int. J. Neuropsychopharmacol. 2, 67–69.

Heisler, L.K., Chu, H.M., Brennan, T.J., Danao, J.A., Bajwa, P., Parsons, L.H., Tecott, L.H., 1998. Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice [see comments]. Proc. Natl. Acad. Sci. U. S. A. 95, 15049–15054.

Hendricks, T., Francis, N., Fyodorov, D., Deneris, E.S., 1999. The ETS domain factor Pet-1 is an early and precise marker of central serotonin neurons and interacts with a conserved element in serotonergic genes. J. Neurosci. 19, 10348–10356.

Hendricks, T.J., Fyodorov, D.V., Wegman, L.J., Lelutiu, N.B., Pehek, E.A., Yamamoto, B., Silver, J., Weeber, E.J., Sweatt, J.D., Deneris, E.S., 2003. Pet-1 ETS gene plays a critical role in 5-HT neuron development and is required for normal anxiety-like and aggressive behavior. Neuron 37, 233–247.

Herdegen, T., Leah, J.D., 1998. Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. Brain Res. Brain Res. Rev. 28, 370–490.

Holmes, A., Murphy, D.L., Crawley, J.N., 2002. Reduced aggression in mice lacking the serotonin transporter. Psychopharmacology (Berl.) 161, 160–167.

Holmes, A., Yang, R.J., Lesch, K.P., Crawley, J.N., Murphy, D.L., 2003. Mice lacking the serotonin transporter exhibit 5-HT1A receptor-mediated abnormalities in tests for anxiety-like behavior. Neuropsychopharmacology (in press).

Hoyer, D., Martin, G., 1997. 5-HT receptor classification and nomenclature: towards a harmonization with the human genome. Neuropharmacology 36, 419–428.

Hynes, M., Ye, W., Wang, K., Stone, D., Murone, M., Sauvage, F., Rosenthal, A., 2000. The seven-transmembrane receptor smoothened cell—autonomously induces multiple ventral cell types. Nat. Neurosci. 3, 41–46.

Karschin, C., Dissmann, E., Stuhmer, W., Karschin, A., 1996. IRK(1–3) and GIRK(1–4) inwardly rectifying K+ channel mRNAs are differentially expressed in the adult rat brain. J. Neurosci. 16, 3559–3570.

Kernie, S.G., Liebl, D.J., Parada, L.F., 2000. BDNF regulates eating behavior and locomotor activity in mice. EMBO J. 19, 1290–1300.

Korte, S.M., Meijer, O.C., de Kloet, E.R., Buwalda, B., Keijser, J., Sluyter, F., van Oortmerssen, G., Bohus, B., 1996. Enhanced 5-HT1A receptor expression in forebrain regions of aggressive house mice. Brain Res. 736, 338–343.

Lappalainen, J., Long, J.C., Eggert, M., Ozaki, N., Robin, R.W., Brown, G.L., Naukkarinen, H., Virkkunen, M., Linnoila, M., Goldman, D., 1998. Linkage of antisocial alcoholism to the serotonin 5-HT1B receptor gene in 2 populations. Arch. Gen. Psychiatry 55, 989–994.

Lauder, J.M., 1993. Neurotransmitters as growth regulatory signals: role of receptors and second messengers. Trends Neurosci. 16, 233–240.

Lebrand, C., Cases, O., Adelbrecht, C., Doye, A., Alvarez, C., Elmestikawy, S., Seif, I., Gaspar, P., 1996. Transient uptake and storage of serotonin in developing thalamic neurons. Neuron 17, 823–835.

Le Poul, E., Laaris, N., Doucet, E., Laporte, A.M., Hamon, M., Lanfumey, L., 1995. Early desensitization of somato-dendritic 5-HT1A autoreceptors in rats treated with fluoxetine or paroxetine. Naunyn Schmiedebergs Arch. Pharmacol. 352, 141–148.

Lerer, B., Gelfin, Y., Gorfine, M., Allolio, B., Lesch, K.P., Newman, M.E., 1999. 5-HT1A receptor function in normal subjects on clinical doses of fluoxetine: blunted temperature and hormone responses to ipsapirone challenge. Neuropsychopharmacology 20, 628–639.

Lesch, K.P., 2001a. Mouse anxiety: the power of knockout. Pharmacogenomics J. 1, 187–192.

Lesch, K.P., 2001b. Variation of serotonergic gene expression: neurodevelopment and the complexity of response to psychopharmacologic drugs. Eur. Neuropsychopharmacol. 11, 457–474.

Lesch, K.P., 2002. Gene–environment interaction in generalized anxiety disorder. In: Nutt, D., Rickels, K., Stein, D. (Eds.), Generalized Anxiety Disorders: Symptomatology, Pathogenesis and Management. Dunitz, London, pp. 71–78.

Lesch, K.P., 2003. Neuroticism and serotonin: a developmental genetic perspective. In: Plomin, R., DeFries, J., Craig, I., McGuffin, P. (Eds.), Behavioral Genetics in the Postgenomic Era. American Psychiatric Press, Washington, DC, pp. 389–423.

Lesch, K.P., Merschdorf, U., 2000. Impulsivity, aggression, and serotonin: a molecular psychobiological perspective. Behav. Sci. Law 18, 581–604.

Lesch, K.P., Mössner, R., 1998. Genetically driven variation in serotonin uptake: is there a link to affective spectrum, neurodevelopmental, and neurodegenerative disorders? Biol. Psychiatry 44, 179–192.

Lesch, K.P., Mössner, R., 1999. 5-HT1A receptor inactivation: anxiety or depression as a murine experience. Int. J. Neuropsychopharmacol. 2, 327–331.

Lesch, K.P., Murphy, D.L., 2003. Molecular genetics of transporters for norepinephrine, dopamine, and serotonin in behavioral traits and complex diseases. In: Broeer, S., Wagner, C.A. (Eds.), Membrane Transport Diseases: Molecular Basis of Inherited Transport Defects. Kluwer Academic Publishing/Plenum, New York (in press).

Lesch, K.P., Disselkamp-Tietze, J., Schmidtke, A., 1990a. 5-HT1A receptor function in depression: effect of chronic amitriptyline treatment. J. Neural Transm. Gen. Sect. 80, 157–161.

Lesch, K.P., Mayer, S., Disselkamp-Tietze, J., Hoh, A., Wiesmann, M., Osterheider, M., Schulte, H.M., 1990b. 5-HT1A receptor responsivity in unipolar depression. Evaluation of ipsapirone-induced ACTH and cortisol secretion in patients and controls. Biol. Psychiatry 28, 620–628.

Lesch, K.P., Hoh, A., Schulte, H.M., Osterheider, M., Muller, T., 1991. Long-term fluoxetine treatment decreases 5-HT1A receptor responsivity in obsessive–compulsive disorder. Psychopharmacology (Berl.) 105, 415–420.

Lesch, K.P., Wiesmann, M., Hoh, A., Muller, T., Disselkamp-Tietze, J., Osterheider, M., Schulte, H.M., 1992. 5-HT1A receptor-effector system responsivity in panic disorder. Psychopharmacology (Berl.) 106, 111–117.

Lesch, K.P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Müller, C.R., Hamer, D.H., Murphy, D.L., 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274, 1527–1531.

Lesch, K.P., Greenberg, B.D., Higley, J.D., Murphy, D.L., 2002. Serotonin transporter, personality, and behavior: toward dissection of gene–gene and gene–environment interaction. In: Benjamin, J., Ebstein, R., Belmaker, R.H. (Eds.), Molecular Genetics and the Human Personality. American Psychiatric Press, Washington, DC, pp. 109–135.

Li, Q., Levy, A.D., Cabrera, T.M., Brownfield, M.S., Battaglia, G., Van de

Kar, L.D., 1993. Long-term fluoxetine, but not desipramine, inhibits the ACTH and oxytocin responses to the 5-HT1A agonist, 8-OH-DPAT, in male rats. Brain Res. 630, 148–156.

Li, Q., Wichems, C., Heils, A., Van De Kar, L.D., Lesch, K.P., Murphy, D.L., 1999. Reduction of 5-hydroxytryptamine (5-HT)(1A)-mediated temperature and neuroendocrine responses and 5-HT(1A) binding sites in 5-HT transporter knockout mice. J. Pharmacol. Exp. Ther. 291, 999–1007.

Li, Q., Wichems, C., Heils, A., Lesch, K.P., Murphy, D.L., 2000. Reduction in the density and expression, but not G-protein coupling, of serotonin receptors (5-HT1A) in 5-HT transporter knock-out mice: gender and brain region differences. J. Neurosci. 20, 7888–7895.

Lopez, J.F., Chalmers, D.T., Little, K.Y., Watson, S.J., 1998. A.E. Bennett Research Award. Regulation of serotonin1A, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus: implications for the neurobiology of depression. Biol. Psychiatry 43, 547–573.

Lundell, M.J., Hirsh, J., 1998. Eagle is required for the specification of serotonin neurons and other neuroblast 7-3 progeny in the *Drosophila* CNS. Development 125, 463–472.

Luscher, C., Jan, L.Y., Stoffel, M., Malenka, R.C., Nicoll, R.A., 1997. G protein-coupled inwardly rectifying K+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. Neuron 19, 687–695.

Lyons, W.E., Mamounas, L.A., Ricaurte, G.A., Coppola, V., Reid, S.W., Bora, S.H., Wihler, C., Koliatsos, V.E., Tessarollo, L., 1999. Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. Proc. Natl. Acad. Sci. U. S. A. 96, 15239–15244.

MacQueen, G.M., Ramakrishnan, K., Croll, S.D., Siuciak, J.A., Yu, G., Young, L.T., Fahnestock, M., 2001. Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression. Behav. Neurosci. 115, 1145–1153.

Maier, D.L., Mani, S., Donovan, S.L., Soppet, D., Tessarollo, L., McCasland, J.S., Meiri, K.F., 1999. Disrupted cortical map and absence of cortical barrels in growth-associated protein (GAP)-43 knockout mice. Proc. Natl. Acad. Sci. U. S. A. 96, 9397–9402.

Mansour-Robaey, S., Mechawar, N., Radja, F., Beaulieu, C., Descarries, L., 1998. Quantified distribution of serotonin transporter and receptors during the postnatal development of the rat barrel field cortex. Dev. Brain Res. 107, 159–163.

Manuck, S.B., Flory, J.D., Ferrell, R.E., Dent, K.M., Mann, J.J., Muldoon, M.F., 1999. Aggression and anger-related traits associated with a polymorphism of the tryptophan hydroxylase gene. Biol. Psychiatry 45, 603–614.

Matise, M.P., Epstein, D.J., Park, H.L., Platt, K.A., Joyner, A.L., 1998. Gli2 is required for induction of floor plate and adjacent cells, but not most ventral neurons in the mouse central nervous system. Development 125, 2759–2770.

Mayorga, A.J., Dalvi, A., Page, M.E., Zimov-Levinson, S., Hen, R., Lucki, I., 2001. Antidepressant-like behavioral effects in 5-hydroxytryptamine(1A) and 5-hydroxytryptamine(1B) receptor mutant mice. J. Pharmacol. Exp. Ther. 298, 1101–1107.

Minichiello, L., Korte, M., Wolfer, D., Kuhn, R., Unsicker, K., Cestari, V., Rossi-Arnaud, C., Lipp, H.P., Bonhoeffer, T., Klein, R., 1999. Essential role for TrkB receptors in hippocampus-mediated learning. Neuron 24, 401–414.

Nestler, E.J., Gould, E., Manji, H., Buncan, M., Duman, R.S., Greshenfeld, H.K., Hen, R., Koester, S., Lederhendler, I., Meaney, M., Robbins, T., Winsky, L., Zalcman, S., 2002. Preclinical models: status of basic research in depression. Biol. Psychiatry 52, 503–528.

New, A.S., Gelernter, J., Yovell, Y., Trestman, R.L., Nielsen, D.A., Silverman, J., Mitropoulou, V., Siever, L.J., 1998. Tryptophan hydroxylase genotype is associated with impulsive-aggression measures: a preliminary study. Am. J. Med. Genet. 81, 13–17.

Nielsen, D.A., Goldman, D., Virkkunen, M., Tokola, R., Rawlings, R., Linnoila, M., 1994. Suicidality and 5-hydroxyindoleacetic acid concentration associated with a tryptophan hydroxylase polymorphism. Arch. Gen. Psychiatry 51, 34–38.

Nielsen, D.A., Virkkunen, M., Lappalainen, J., Eggert, M., Brown, G.L., Long, J.C., Goldman, D., Linnoila, M., 1998. A tryptophan hydroxylase gene marker for suicidality and alcoholism. Arch. Gen. Psychiatry 55, 593–602.

Ninan, P.T., 1999. The functional anatomy, neurochemistry, and pharmacology of anxiety. J. Clin. Psychiatry 60 (Suppl. 22), 12–17.

Olivier, B., Miczek, K.A., 1999. Fear and anxiety: mechanisms, models and molecules. In: Dodman, N., Shuster, I. (Eds.), Psychopharmacology of Animal Behavior Disorders. Blackwell, London, pp. 105–121.

Olivier, B., Soudijn, W., van Wijngaarden, I., 1999. The 5-HT1A receptor and its ligands: structure and function. Prog. Drug Res. 52, 103–165.

Olivier, B., Pattij, T., Wood, S.J., Oosting, R., Sarnyai, Z., Toth, M., 2001. The 5-HT(1A) receptor knockout mouse and anxiety. Behav. Pharmacol. 12, 439–450.

Parks, C.L., Robinson, P.S., Sibille, E., Shenk, T., Toth, M., 1998. Increased anxiety of mice lacking the serotonin1A receptor. Proc. Natl. Acad. Sci. U. S. A. 95, 10734–10739.

Parsons, L.H., Kerr, T.M., Tecott, L.H., 2001. 5-HT(1A) receptor mutant mice exhibit enhanced tonic, stress-induced and fluoxetine-induced serotonergic neurotransmission. J. Neurochem. 77, 607–617.

Pawlak, R., Magarinos, A.M., Melchor, J., McEwen, B., Strickland, S., 2003. Tissue plasminogen activator in the amygdala is critical for stress-induced anxiety-like behavior. Nat. Neurosci. 6, 168–174.

Persico, A.M., Revay, R.S., Mössner, R., Conciatori, M., Marino, R., Baldi, A., Cabib, S., Pascucci, T., Sora, I., Uhl, G.R., Murphy, D.L., Lesch, K.P., Keller, F., 2001. Barrel pattern formation in somatosensory cortical layer IV requires serotonin uptake by thalamocortical endings, while vesicular monoamine release is necessary for development of supragranular layers. J. Neurosci. 21, 6862–6873.

Persico, A.M., Baldi, A., Dell'Acqua, M.L., Moessner, R., Murphy, D.L., Lesch, K.P., Keller, F., 2003. Reduced programmed cell death in brains of serotonin transporter knockout mice. NeuroReport 14, 341–344.

Phillips, T.J., Huson, M., Gwiazdon, C., Burkhart-Kasch, S., Shen, E.H., 1995. Effects of acute and repeated ethanol exposures on the locomotor activity of BXD recombinant inbred mice. Alcohol., Clin. Exp. Res. 19, 269–278.

Ramboz, S., Oosting, R., Amara, D.A., Kung, H.F., Blier, P., Mendelsohn, M., Mann, J.J., Brunner, D., Hen, R., 1998. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc. Natl. Acad. Sci. U. S. A. 95, 14476–14481.

Rebsam, A., Seif, I., Gaspar, P., 2002. Refinement of thalamocortical arbors and emergence of barrel domains in the primary somatosensory cortex: a study of normal and monoamine oxidase a knock-out mice. J. Neurosci. 22, 8541–8552.

Reif, A., Lesch, K.P., 2003. Toward a molecular architecture of personality. Behav. Brain Res. 139, 1–20.

Richer, M., Hen, R., Blier, P., 2002. Modification of serotonin neuron properties in mice lacking 5-HT1A receptors. Eur. J. Pharmacol. 435, 195–203.

Rios, M., Fan, G., Fekete, C., Kelly, J., Bates, B., Kuehn, R., Lechan, R.M., Jaenisch, R., 2001. Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. Mol. Endocrinol. 15, 1748–1757.

Rothe, C., Gutknecht, L., Freitag, C., Tauber, R., Franke, P., Fritze, J., Wagner, G., Peikert, G., Wenda, B., Sand, P., Jacob, C., Rietschel, M., Nöthen, M., Garritsen, H., Fimmers, R., Deckert, J., Lesch, K.P., 2003. Association of a functional −1019C>G 5-HT1A receptor gene polymorphism with panic disorder with agoraphobia. Int. J. Neuropsychopharmacol. (in press).

Salichon, N., Gaspar, P., Upton, A.L., Picaud, S., Hanoun, N., Hamon, M., De Maeyer, E.E., Murphy, D.L., Mossner, R., Lesch, K.P., Hen, R., Seif, I., 2001. Excessive activation of serotonin (5-HT) 1B receptors disrupts the formation of sensory maps in monoamine oxidase A and 5-HT transporter knock-out mice. J. Neurosci. 21, 884–896.

Samochowiec, J., Lesch, K.P., Rottmann, M., Smolka, M., Syagailo, Y.V., Okladnova, O., Rommelspacher, H., Winterer, G., Schmidt, L.G., Sander, T., 1999. Association of a regulatory polymorphism in the promoter region of the monoamine oxidase A gene with antisocial alcoholism [in process citation]. Psychiatry Res. 86, 67–72.

Santarelli, L., Gobbi, G., Debs, P.C., Sibille, E.L., Blier, P., Hen, R., Heath, M.J.S., 2001. Genetic and pharmacological disruption of neurokinin 1 receptor function decreases anxiety-related behaviors and increases serotonergic function. Proc. Natl. Acad. Sci. U. S. A. 98, 1912–1917.

Sargent, P., Williamson, D.J., Pearson, G., Odontiadis, J., Cowen, P.J., 1997. Effect of paroxetine and nefazodone on 5-HT1A receptor sensitivity. Psychopharmacology (Berl.) 132, 296–302.

Sargent, P.A., Kjaer, K.H., Bench, C.J., Rabiner, E.A., Messa, C., Meyer, J., Gunn, R.N., Grasby, P.M., Cowen, P.J., 2000. Brain serotonin1A receptor binding measured by positron emission tomography with [11C]WAY-100635: effects of depression and antidepressant treatment. Arch. Gen. Psychiatry 57, 174–180.

Saudou, F., Amara, D.A., Dierich, A., LeMeur, M., Ramboz, S., Segu, L., Buhot, M.C., Hen, R., 1994. Enhanced aggressive behavior in mice lacking 5-HT1B receptor. Science 265, 1875–1878.

Schachner, M., 1997. Neural recognition molecules and synaptic plasticity. Curr. Opin. Cell Biol. 9, 627–634.

Schaefer, M.L., Wong, S.T., Wozniak, D.F., Muglia, L.M., Liauw, J.A., Zhuo, M., Nardi, A., Hartman, R.E., Vogt, S.K., Luedke, C.E., Storm, D.R., Muglia, L.J., 2000. Altered stress-induced anxiety in adenylyl cyclase type VIII-deficient mice. J. Neurosci. 20, 4809–4820.

Schuman, E.M., 1999. Neurotrophin regulation of synaptic transmission. Curr. Opin. Neurobiol. 9, 105–109.

Seif, I., De Maeyer, E., 1999. Knockout corner—knockout mice for monoamine oxidase A. Int. J. Neuropsychopharmacol. 2, 241–243.

Sibille, E., Pavlides, C., Benke, D., Toth, M., 2000. Genetic inactivation of the Serotonin(1A) receptor in mice results in downregulation of major GABA(A) receptor alpha subunits, reduction of GABA(A) receptor binding, and benzodiazepine-resistant anxiety. J. Neurosci. 20, 2758–2765.

Sora, I., Wichems, C., Takahashi, N., Li, X.F., Zeng, Z., Revay, R., Lesch, K.P., Murphy, D.L., Uhl, G.R., 1998. Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. Proc. Natl. Acad. Sci. U. S. A. 95, 7699–7704.

Sora, I., Hall, F.S., Andrews, A.M., Itokawa, M., Li, X.F., Wei, H.B., Wichems, C., Lesch, K.P., Murphy, D.L., Uhl, G.R., 2001. Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. Proc. Natl. Acad. Sci. U. S. A. 98, 5300–5305.

Spenger, C., Hyman, C., Studer, L., Egli, M., Evtouchenko, L., Jackson, C., Dahl-Jorgensen, A., Lindsay, R.M., Seiler, R.W., 1995. Effects of BDNF on dopaminergic, serotonergic, and GABAergic neurons in cultures of human fetal ventral mesencephalon. Exp. Neurol. 133, 50–63.

Stark, K.L., Hen, R., 1999. Knockout corner—5-HT1B receptor knockout mice: a review. Int. J. Neuropsychopharmacol. 2, 145–150.

Stork, O., Welzl, H., Wotjak, C.T., Hoyer, D., Delling, M., Cremer, H., Schachner, M., 1999. Anxiety and increased 5-HT1A receptor response in NCAM null mutant mice. J. Neurobiol. 40, 343–355.

Strobel, A., Gutknecht, L., Rothe, C., Zheng, Y., Reif, A., Brocke, B., Lesch, K.P., 2003. Allelic variation of serotonin receptor 1A function is associated with anxiety- and depression-related traits. J. Neurol. Transm. (in press).

Tecott, L.H., Barondes, S.H., 1996. Genes and aggressiveness. Behavioral genetics. Curr. Biol. 6, 238–240.

Toth, M., 2003. 5-HT(1A) receptor knockout mouse as a genetic model of anxiety. Eur. J. Pharmacol. 463, 177–184.

Upton, A.L., Salichon, N., Lebrand, C., Ravary, A., Blakely, R., Seif, I., Gaspar, P., 1999. Excess of serotonin (5-HT) alters the segregation of ispilateral and contralateral retinal projections in monoamine oxidase A knock-out mice: possible role of 5-HT uptake in retinal ganglion cells during development. J. Neurosci. 19, 7007–7024.

Upton, A.L., Ravary, A., Salichon, N., Moessner, R., Lesch, K.P., Hen, R., Seif, I., Gaspar, P., 2002. Lack of 5-HT(1B) receptor and of serotonin transporter have different effects on the segregation of retinal axons in the lateral geniculate nucleus compared to the superior colliculus. Neuroscience 111, 597–610.

van Doorninck, J.H., van Der Wees, J., Karis, A., Goedknegt, E., Engel, J.D., Coesmans, M., Rutteman, M., Grosveld, F., De Zeeuw, C.I., 1999. GATA-3 is involved in the development of serotonergic neurons in the caudal raphe nuclei. J. Neurosci. 19, RC12.

Vitalis, T., Cases, O., Gillies, K., Hanoun, N., Hamon, M., Seif, I., Gaspar, P., Kind, P., Price, D.J., 2002. Interactions between TrkB signaling and serotonin excess in the developing murine somatosensory cortex: a role in tangential and radial organization of thalamocortical axons. J. Neurosci. 22, 4987–5000.

Walther, D.J., Peter, J.U., Bashammakh, S., Hortnagl, H., Voits, M., Fink, H., Bader, M., 2003. Synthesis of serotonin by a second tryptophan hydroxylase isoform. Science 299, 76.

Weiss, S.M., Lightowler, S., Stanhope, K.J., Kennett, G.A., Dourish, C.T., 2000. Measurement of anxiety in transgenic mice. Rev. Neurosci. 11, 59–74.

Wischmeyer, E., Doring, F., Spauschus, A., Thomzig, A., Veh, R., Karschin, A., 1997. Subunit interactions in the assembly of neuronal Kir3.0 inwardly rectifying K+ channels. Mol. Cell. Neurosci. 9, 194–206.

Wisor, J.P., Wurts, S.W., Hall, F.S., Lesch, K.P., Murphy, D.L., Uhl, G.R., Edgar, D.M., 2003. Altered rapid eye movement sleep timing in serotonin transporter knockout mice. NeuroReport 14, 233–238.

Wissink, S., Meijer, O., Pearce, D., van Der Burg, B., van Der Saag, P.T., 2000. Regulation of the rat serotonin-1A receptor gene by corticosteroids. J. Biol. Chem. 275, 1321–1326.

Ye, W., Shimamura, K., Rubenstein, J.L., Hynes, M.A., Rosenthal, A., 1998. FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. Cell 93, 755–766.

Zhou, J., Iacovitti, L., 2000. Mechanisms governing the differentiation of a serotonergic phenotype in culture. Brain Res. 877, 37–46.

Zhuang, X., Gross, C., Santarelli, L., Compan, V., Trillat, A.C., Hen, R., 1999. Altered emotional states in knockout mice lacking 5-HT1A or 5-HT1B receptors. Neuropsychopharmacology 21, 52S–60S.

Zubenko, G.S., Hughes, H.B., Stiffler, J.S., Brechbiel, A., Zubenko, W.N., Maher, B.S., Marazita, M.L., 2003. Sequence variations in CREB1 cosegregate with depressive disorders in women. Mol. Psychiatry 8, 611–618.