ARTICLE IN PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT

ELSEVIER

Neuroscience and Biobehavioral Reviews ▮ (▮▮▮▮) ▮▮▮–▮▮▮

NEUROSCIENCE AND BIOBEHAVIORAL REVIEWS

www.elsevier.com/locate/neubiorev

Review

Altered brain activity processing in high-anxiety rodents revealed by challenge paradigms and functional mapping

Nicolas Singewald*

Department of Pharmacology & Toxicology, Institute of Pharmacy and Center of Molecular Biosciences, University of Innsbruck, A-6020 Innsbruck, Austria

Received 28 November 2005; received in revised form 10 February 2006; accepted 15 February 2006

Abstract

Pathological anxiety involves aberrant processing of emotional information that is hypothesized to reflect perturbations in fear/anxiety pathways. The affected neurobiological substrates in patients with different anxiety disorders are just beginning to be revealed. Important leads for this research can be derived from findings obtained in psychopathologically relevant rodent models of enhanced anxiety, by revealing where in the brain neuronal processing in response to diverse challenges is different to that in animals with lower anxiety levels. Different functional mapping methods in various rodent models, including psychogenetically selected lines or genetically modified animals, have been used for this purpose. These studies show that the divergent anxiety-related behavioral response of high-anxiety- vs. normal and/or low-anxiety rodents to emotional challenges is associated with differential neuronal activation in restricted parts of proposed fear/anxiety circuitries including brain areas thought to be important in stress, emotion and memory. The identification of neuronal populations showing differential activation depends in part on the applied emotional challenge, indicating that specific facets of elicited fear or anxiety preferentially engage particular parts of the fear/anxiety circuitry. Hence, only the use of an array of different challenges will reveal most affected brain areas. A number of the neuronal substrates identified are suggested as candidate mediators of dysfunctional brain activation in pathological anxiety. Indeed, key findings revealed in these rodent models show parallels to observations in human symptom provocation studies comparing anxiety disorder patients with healthy volunteers. Work to investigate exactly which of the changed neuronal activation patterns in high-anxiety rodents has to be modulated by therapeutic drugs to achieve effective anxiolysis and via which neurochemical pathways this can be accomplished is at its early stages but has identified a small number of promising candidates. Extending these approaches should help to provide further insight into these mechanisms, revealing new leads for therapeutic targets and strategies.

Keywords: Functional imaging; Pathological anxiety; Fear circuitry; fMRI; Immediate early gene expression; Fos; Symptom provocation; Trait anxiety

Contents

*Tel.: +43 512 507 5608; fax: +43 512 507 2931.
E-mail address: nicolas.singewald@uibk.ac.at.

0149-7634/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neubiorev.2006.02.003

1. Introduction

Fear and anxiety as normal reactions to threatening situations/stressors are considered to be common internal emotional states in the human population, serving physiological protective functions that, for example, help to cope with a stressor. Pathological anxiety, on the other hand, is characterized by an excessive experience of anxiety. This represents the core symptom in anxiety disorders which cause significant distress and suffering in the lives of affected patients. Fear behaviors, which occur in response to explicit, imminent threats are usually short-lived, evoking intense escape attempts and avoidance of the threat. Anxiety behaviors occur in response to less explicit, more generalized threats and maintain attentiveness by modulating arousal and risk assessment (see, e.g. Griebel et al., 1995). Both disturbed fear and anxiety responses play a role in anxiety disorders and although it is out of question that there are differences between fear and anxiety, they are processed in greatly overlapping neurocircuitries and therefore the distinction between fear and anxiety is not always emphasized in this review.

Anxiety disorders are among the most common types of psychiatric disorder and encompass several different disorders, which are characterized by a diverse range of symptoms triggered in a variety of situations. Anxiety-related illness constitutes a major burden to western countries as it is estimated that around 15% of the population are affected each year (Fichter et al., 1996; Kessler et al., 1994; Narrow et al., 2002). Lifetime prevalence rates for all anxiety disorders are around 19% for men and 31% for women (Bremner, 2004). The diagnostic systems DSM-IV (American Psychiatric Association, 1994) and ICD-10 (World Health Organization, 1992) describe several distinct human anxiety disorders, including panic disorder, social phobia, specific phobia, post-traumatic stress disorder (PTSD), generalized anxiety disorder (GAD) and obsessive–compulsive disorder. Despite extensive research efforts and considerable advances in the understanding of the neurobiological features of anxiety disorders (Millan, 2003), the exact mechanisms underlying anxiety disorders as well as their effective treatment remain incompletely understood. Therefore, the identification of neuronal structures and circuits mediating anxiety disorders continues to be an essential step toward understanding the mechanisms that contribute to pathological fear and anxiety (Shekhar et al., 2001). Since anxiety disorders differ in a number of features, including symptoms, age of onset, prevalence in males and females, and treatment response, it may be suggested that in each syndrome a unique composite circuit may be important. Exactly mimicking these different anxiety syndromes in animal anxiety models and tests seems a very difficult, if not impossible, task (Cryan and Holmes, 2005; Kalueff and Tuohimaa, 2004; Shekhar et al., 2001). However, a considerable overlap exists of neuroanatomical structures and pathways involved in the processing of anxiety in different anxiety disorders (Cannistraro and Rauch, 2003; Pratt, 1992; Sandford et al., 2000) and there are specific symptoms that can be induced in animals and humans that are thought to be a common thread across anxiety disorders (Shekhar et al., 2001). It is well accepted that in pathological anxiety a combination of internal (genetic) and external (environmental) factors leads to enhanced vulnerability, for example, to respond to challenges adequately. There is evidence that dysfunctions in specific neurocircuitries in the brain underlie inadequate responding to (aversive) emotional challenges, including stressors, resulting in exaggerated and/or prolonged fear responses, which are typical signs in a number of anxiety disorders. Not surprisingly, stress is known to be an important triggering factor in anxiety disorders but also in such disorders as depression and schizophrenia. In experimental anxiety research, mostly aversive emotional challenges including stressors are therefore used in symptom provocation imaging studies to reveal sites of dysfunctional neuronal processing in the brains of patients displaying pathological anxiety (see below and Section 4.1). This research has opened new directions and perspectives regarding ways to correlate aberrant fear/anxiety responses with changes in activity of specific neurons in individual brain regions. Since anxiety as a biologically essential emotion is highly conserved during evolution, animal models are thus considered powerful tools to guide and complement research efforts in humans, with several advantages but also disadvantages. The advantages include the possibility of studying higher numbers of individuals with a more homogenous genetic background and more

controlled stress history, as well as of investigating the influence of genetic manipulations on the function of circuits and the emotional behaviors they mediate. While insufficient modeling of the influence of higher cortical functions may be a potential disadvantage. The experimental strategy used increasingly in human studies, i.e. exposing individuals with excessive levels of anxiety to emotional challenges and revealing sites of dysfunctional neuronal processing (for review, see Anand and Shekhar, 2003; Bremner, 2004; Cannistraro and Rauch, 2003; Charney, 2003; Fredrikson and Furmark, 2003; Malizia, 1999; Rauch et al., 1997) can also be applied in animals. Animal models for trait anxiety, which can closely mirror important features of the human psychopathology, seem to be particularly suited for such investigation. The status of this research in rodents is summarized in this review.

2. Functional imaging in rodents: techniques used in anxiety research

A number of methods have been developed to simultaneously monitor neuronal activity patterns in response to physiological and pathophysiological conditions in large parts of the rodent brain. Most of the methods available both in animals and humans (Kessler, 2003) make use of indirect measures of neuronal activity such as blood flow or glucose metabolism. The small size of the rodent brain compared with the human brain is only one particular additional technical challenge for these methods in animal work. An electrophysiological technique allowing global monitoring of neuronal activity is electroencephalography (EEG), which provides, in contrast to all other mapping methods (see below), a direct measure of neuronal activation. Excellent (real time) temporal resolution (10–100 ms) and the possibility of studying conscious animals are particular advantages of such methods. The major disadvantage for anxiety research, however, is that the spatial resolution is very poor and only large (superficial) regions can be evaluated. Sufficient spatial resolution is a requisite for identification of defined neuronal populations exhibiting altered neuronal activity in response to a given stimulus. The currently most widely used functional imaging methods in humans—single photon emission computed tomography (SPECT), positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), which make use of the relationship between neuronal activity and cerebral blood flow (CBF) (Gotoh et al., 2001; Mintun et al., 2001), blood oxygen level dependent (BOLD) signals (Arthurs and Boniface, 2002), and glucose utilization (Sokoloff, 1999) are also available for rodents and provide temporal resolutions ranging between a few seconds and several minutes. For example, fMRI can image the brain in seconds (or faster, see Menon et al., 1998), while a fluorodeoxyglucose (FDG) PET scan sums activity over minutes. Although the small size of the brain renders investigation of small brain areas extremely difficult, the spatial resolution of some of these approaches, such as high-resolution fMRI (e.g. Lu et al., 2004), is impressive in the meantime. However, anesthesia is necessary in most cases to sufficiently restrain animals in the scanner and to minimize motion artifacts (Lukasik and Gillies, 2003), which limits the utility of these methods for anxiety research in conscious animals. A few attempts have been made to use fMRI in conscious rodents, trying to reduce stress associated with the restraining procedure by training or administration of anxiolytic drugs (Lahti et al., 1998; Sachdev et al., 2003), but the utility of such approaches for anxiety research remains to be established.

Due to the shortcomings of the above-mentioned methods, functional staining techniques providing excellent (single-cell) spatial resolution, but limited temporal resolution (Chaudhuri et al., 1997; Guzowski et al., 2005), are frequently applied in stress and anxiety research to investigate neuronal activity patterns in widespread brain areas. These methods have exploited the observation that neurons respond to increased activity by changing glucose metabolism and levels of gene expression (Chaudhuri, 1997): thus, 2-deoxyglucose or FDG mapping (Barrett et al., 2003; Pratt, 1992), regional CBF-related tissue radioactivity (Holschneider et al., 2005) and expression of immediate early genes (IEGs), visualized by in situ hybridization, autoradiography or immunohistochemistry, are mainly used for stress/anxiety research. Measurement of cytochrome oxidase (Wong-Riley, 1989), a rate-limiting mitochondrial enzyme for oxidative energy metabolism of ATP, has also been used as a measure of neuronal activation in this field (e.g. Poremba et al., 1998), although this method seems to require longer averaging of cerebral activation than IEG expression approaches. Since the induction of various IEGs is proposed to correlate with their functional activation in widespread neurons (for review, see Hoffman and Lyo, 2002; Herrera and Robertson, 1996; Morgan et al., 1987; Sagar et al., 1988; Sheng and Greenberg, 1990), monitoring the expression of IEG genes, including c-*fos*, *zif268* (Krox-24, Egr1, NGFI, ZENK), and others, has been particularly useful in identifying brain areas involved in emotional processes, stress and fear/anxiety states (Brandao et al., 2005; Chan et al., 1993; Cullinan et al., 1995; Dielenberg and McGregor, 2001; Duncan et al., 1996a; Keay and Bandler, 2001; Kovacs, 1998; Malkani and Rosen, 2000; Martinez et al., 2002; Pacak and Palkovits, 2001; Ribeiro-Barbosa et al., 2005; Sandner et al., 1993; Senba and Ueyama, 1997). IEG expression analysis is conducted post mortem and since no preparatory procedures are necessary, as they are in PET, fMRI and electrophysiological techniques, animals are enabled to behave in a more natural and unrestricted way in the studied environments.

Of the IEGs, c-fos has been most widely used. This is for several reasons, including (i) low levels of basal expression, for example (Herdegen et al., 1995), making its upregulation readily detectable, (ii) broad dynamic range of mRNA and protein levels and (iii) short half-life of both mRNA and protein. c-fos mRNA is induced within a couple of

minutes upon a wide range of stimuli and peaks between 20 and 60 min (Cullinan et al., 1995; Ikeda et al., 1994). The maximal level of the protein product of c-fos, c-Fos, can be detected between 1 and 3 h following an acute challenge; then, 4–6 h after treatment, it gradually disappears from the cell nucleus (Duncan et al., 1996b; Kovacs and Sawchenko, 1996; Zangenehpour and Chaudhuri, 2002). Additionally, the fact that the c-Fos protein is nuclear and can be detected immunohistochemically (Cecchi et al., 1999) permits double- or multiple labeling to identify the phenotype and receptor endowment of the activated neuron (Ceccatelli et al., 1989). For more comprehensive neuroanatomical studies, the combination with tract-tracing procedures is possible. c-Fos is a member of the AP-1 (activator protein-1) family of inducible transcription factors and binds to regulatory DNA sequences with a Jun family member as a heterodimer. The AP-1 transcription factor can activate (repress) transcription of late-response genes and its binding site is found in a large number of promoters (for review, see Hoffman and Lyo, 2002). Although the downstream targets of AP-1 (e.g. genes encoding tyrosine hydroxylase, glutamate decarboxylase, proenkephalin, prodynorphin, various receptors and ion channels) are of considerable interest (Herdegen and Leah, 1998) for many reasons, including, for example, for investigating the question of how expression of these genes can be translated into changes in neuronal excitability, plastic changes and finally psychopathology, the discussion of these mechanisms is beyond the scope of this review (for further reading see, for example, Gass et al., 2004; Guzowski, 2002; McClung et al., 2004).

It was observed as early as the 1980s that experimental procedures and treatments presumed to induce and prolong neuronal activation, such as electrical stimulation, seizures, tissue injury, morphine withdrawal, stressors and numerous pharmacological agents, all induce Fos expression in various brain regions (for review, see Dragunow and Faull, 1989; Hoffman and Lyo, 2002; Kovacs, 1998; Morgan and Curran, 1991) indicating an association of increased neuronal activity with the induction of Fos (e.g. Dragunow and Faull, 1989). c-Fos (and also Zif268), which represents the integral of excitatory stimulation-induced postsynaptic neuronal activity, is induced by calcium influx into the cell following stimulation either through voltage-sensitive calcium channels or through *n*-methyl-D-aspartate (NMDA) receptors (Ghosh et al., 1994). Moreover, a wide number of different receptors, when activated, lead to c-fos induction, for example via increase in intracellular cyclic AMP levels and phosphorylation of CREB (Thompson et al., 1995). Indeed, these mechanisms are frequently engaged by stimuli that evoke increased firing in the target neuron. These features form the basis of using Fos as a marker for stimulated neuron activity and render this cellular IEG the most widely used functional anatomical mapping tool to identify cells and extended circuitries that become activated in response to a wide range of stimuli (Ceccatelli et al., 1989; Greenberg and Ziff, 1984; Sagar et al., 1988). Interestingly, it has been shown in rodents that neuronal activation patterns detected in parallel either by Fos expression or by high-resolution fMRI correlated well (e.g. Lu et al., 2004; Lawrence et al., 2004; Lazovic et al., 2005), further supporting the utility of Fos mapping to determine changes in neuronal activation. However, there are some potential shortcomings associated with the Fos methodology that must be taken into account when designing experiments (Chaudhuri and Zangenehpour, 2002; Hoffman and Lyo, 2002). (1) It is important to keep in mind that like most functional imaging methods, including fMRI and PET (see above), Fos expression is a indirect marker of neuronal activation. It is the changes in signal transduction pathways, which induce *c-fos* expression, not depolarization per se. There are instances where Fos induction can be evoked under conditions that are not associated with changes in neuronal firing (stimulatory or inhibitory). For example, the SRE-dependent induction of c-fos can be triggered by the action of growth factors and mitogens (for details, see Hoffman and Lyo, 2002). (2) Conversely, neuronal firing may not always be associated with Fos expression in every neuron in the brain (Hoffman and Lyo, 2002). Thus, absence of c-fos induction does not necessarily indicate a lack of neuronal activity; it may, rather, be due to the fact that some neurons do not express c-fos, or that the thresholds for c-fos induction may be higher in specific neurons. Since essentially all cells express one IEG or another, IEGs different to c-fos may be primarily involved in neuronal activation in these instances. For example, one such brain area may be the ventral tegmental area where higher thresholds to induce c-fos or egr expression by stressors were suspected (Koya et al., 2005). Complementary methods involving simultaneous investigation of additional IEGs (Cullinan et al., 1995; Koya et al., 2005), other adjunct markers (e.g. inducible structural proteins such as Arc; Lyford et al., 1995; Guzowski et al., 2001) or electrophysiological methods should be tested in these cases before conclusive interpretation. (3) Owing to the fact that basal expression of Fos is generally low in most brain areas, there is a lack of response of Fos to inhibitory mechanisms under baseline (unstimulated) conditions. (4) Due to the poor temporal resolution, small and or fast/transient changes in activation may not be picked up by the Fos mapping technique. Despite these potential shortcomings, however, the expression of c-fos, with adequate controls, is a valuable tool to elucidate neuronal pathways that are responsive to specific stimuli and thus participate in respective functional neuronal networks.

3. High-anxiety rodent models

Pathological anxiety is characterized by inappropriate expression of anxiety-related behavior consisting of 'diffuse' hyper-anxiety under basal conditions, a bias to interpret ambiguous situations as threatening, increased avoidance of situations that are perceived to be harmful,

and/or exaggerated reactions to threat (Wood and Toth, 2001). It is still a matter of debate whether 'pathological' anxiety evolves as a quantitative variation of a normal state, i.e. an excess of 'normal' anxiety, or whether there is a qualitative difference (Rosen and Schulkin, 1998). Although the spectrum of emotions experienced by rodents is thought to be much less complex than that in humans, anxiety-related behaviors in particular may be modeled well in rodents and show impressive similarities with human anxiety (Blanchard et al., 2001). Rodent tests of anxiety (models of 'state' anxiety) are frequently used to evaluate putative anxiolytic compounds and to study neurobiological mechanisms underlying anxiety (for review, see Rodgers and Dalvi, 1997; Crawley, 1999; Cryan and Holmes, 2005; Finn et al., 2003). However, most of these anxiety tests evaluate the rodent's normal fear/anxiety reaction, but human pathological anxiety is the reflection of an inappropriate response to a real or perceived stimulus (see Introduction). Therefore, using rodent strains in which the response to challenging (aversive) stimuli is exaggerated, as it is in anxiety-disorder patients, would be more appropriate in such anxiety-related studies. Several approaches have been pursued to mimic long-term, 'pathological' anxiety in rodents. Most commonly used models for this purpose are rodents that were selected for enhanced emotional reactivity or mice with targeted mutations which exhibit phenotypic changes indicative of increased anxiety. Animal models of 'pathological' anxiety are often referred to as 'trait' anxiety models. Unlike 'state' anxiety, 'trait' anxiety does not vary from moment to moment and is considered to be an enduring feature of an individual (for review, see Landgraf and Wigger, 2003; Belzung and Griebel, 2001; Landgraf and Wigger, 2002; Millan, 2003).

3.1. Models developed by selective breeding

The goal of selective breeding of animals is to model (an aspect of) a disorder that is believed to depend upon genetic variation in the human population. The expectation is that after many generations of selective breeding, the high and low lines of a relevant trait or phenotype will be 'enriched' by genes facilitating the corresponding phenotype. Selective breeding for anxiety-related characteristics has been used to generate animals that express particular feature(s) of interest with a higher probability and/or to a greater extent (for review, see Broadhurst, 1960; Clement et al., 2002; Driscoll et al., 1998; Landgraf and Wigger, 2002; Ramos and Mormede, 1998; Steimer and Driscoll, 2005), enhancing the probability of finding anxiety-related neurobiological differences between the lines. Thus, compared with unselected animals, this strategy has major advantages for studying, in particular, mechanisms of exaggerated (or reduced) anxiety-related behavior. Various lines differing in certain aspects of emotionality, including anxiety, such as the Maudsley Reactive and Maudsley Nonreactive strains, Roman, Syracuse rat lines, Tsukuba, Floripa lines, lines with infantile high and low ultrasonic vocalizations, as well as High Anxiety Behavior (HAB) and Low Anxiety Behavior (LAB) rodents, have been generated by using different selection criteria founded around standard anxiety paradigms (Table 1). Many of these selected rodents exhibit a significant increase in anxiety-related behavior as evaluated in different anxiety tests (Table 1), although some of the findings were not always consistent (see, e.g. Blizard and Adams, 2002). Interestingly, many of these more 'emotional' or 'anxious' lines are characterized by a passive coping style and show an enhanced 'depression-like' phenotype in the animal models of depression, such as forced swim test (Abel et al., 1992; Kromer et al., 2005; Liebsch et al., 1998; Zimmerberg et al., 2005) or tail suspension test (Kromer et al., 2005), providing support for current hypotheses that depression and anxiety have important genetic overlap (e.g. Gorwood, 2004), explaining the extensive comorbidity of these disorders (Cryan and Holmes, 2005; Kendler et al., 1992; Talbot, 2004). Unfortunately, so far only one of these models, that of the HAB rodents, has been investigated in functional mapping studies (see Section 4). Therefore, this model will be discussed in more detail.

Since 1993, two Wistar rat lines were selectively bred for high (HAB) and low (LAB) anxiety-related behavior on the Elevated Plus Maze (EPM) at the Max Planck Institute of Psychiatry in Munich (for review, see Landgraf and Wigger, 2002). These lines proved to be extremely divergent in their innate anxiety as revealed in different behavioral tests that were conducted in addition to the EPM, including the open field, light/dark, hole board, social interaction and maternal separation-associated neonate ultrasound vocalization tests (Liebsch et al., 1998; Ohl et al., 2001; Wigger et al., 2001). Comprehensive studies have demonstrated that the selection of HABs and LABs is based on anxiety-related rather than on locomotor behavior (for review, see Landgraf and Wigger, 2002). For example, discriminant functional analysis revealed that, in the light/dark test, the parameters most important for the discrimination between the lines are clearly anxiety-related behaviors. In the EPM test, the most important parameter in this context is percentage of time spent on the open arms, the index that is generally seen as the one that is most closely related to anxiety (File, 2001; Rodgers and Dalvi, 1997; Rodgers and Johnson, 1995). In both tests, locomotor activity turned out to have no significant influence on the discrimination between the two lines (Henniger et al., 2000). Moreover, when singly housed HAB and LAB animals were tested under baseline conditions in their home cages with a radiotelemetric system, no difference in locomotion was found (Liebsch et al., 1998; Muigg and Singewald, unpublished). Another major issue in behavioral studies is the stability of the phenotype. Discrepancies in the behavioral patterns of mice or rats in the same anxiety tests performed in different laboratories have been reported (e.g. Crabbe et al., 1999; Clement et al., 2002). The explanations given for such discrepancies in behavioral

Table 1
Differential anxiety-like behavior in rodents by selective breeding approaches based on anxiety-related tests and parameters

Model name	Selection criterion	Enhanced anxiety verified in	Reference
Rats			
Maudsley Reactive vs. Nonreactive lines	OFD	OFD, OF, L/D, staircase test, EPM (inconsistent)	Review: (Blizard and Adams, 2002)
Roman low vs. high avoidance	Avoidance (shuttle box)	EPM, OF, L/D, L/D-OF	Review (Steimer and Driscoll, 2003; Steimer and Driscoll, 2005)
		FPS	(Yilmazer-Hanke et al., 2002)
Syracuse low- vs. high avoidance rats	Avoidance (shuttle box)	OFD, CTA, PAV CER, and others	Review (Brush, 2003):
Tsukuba high vs. low emotional line	Runway test	Defecation, OF, I-maze	Review: (Fujita et al., 1994; Kitaoka and Fujita, 1991)
		USV (of pups)	(Naito et al., 2000)
HAB vs. LAB	EPM	EPM, OF, L/D, SD, mod. HB, SI, USV(pups)	Review (Landgraf and Wigger, 2002)
		OA	Salome et al., 2004)
Floripa L vs. Floripa H rat line	Locomotion in the central area of an OF	OF, EPM, L/D	(Ramos et al., 2003)
Infantile high- vs. low USV	USV	USV, EPM (inconsistent)	Review (Brunelli, 2005; Dichter et al., 1996)
		SI, OF, emergence	(Zimmerberg et al., 2005)
Mice			
HAB-M vs. LAB-M	EPM	EPM, OA, USV, L/D	(Kromer et al., 2005)

CER, conditioned suppression; CTA, conditioned taste aversion; EPM, elevated plus maze; FPS, fear-potentiated start test; L/D, light dark test; L/D-OF; light dark test—open field; mod. HB, modified holebord test; OF, open field; OFD, open field defecation; PAV, passive avoidance learning; SD, social defeat; SI, social interaction test; USV, ultrasonic vocalization.

studies usually include slight modifications of the testing conditions, differences in the types of parameters scored and also different environmental conditions (Clement et al., 2002). It has been demonstrated in different laboratories that the divergence in anxiety-related behavior between HAB and LAB rats was reliable and independent of the laboratory in which the tests are conducted (Salome et al., 2002). The differences in anxiety-related behavior were highly consistent in all tests performed to date. Together with detected neuroendocrinological abnormalities, these observations have demonstrated that the hyperanxiety in HABs represents a constant and robust trait, which resembles that of psychiatric patients in many aspects (Keck et al., 2002; Liebsch et al., 1998). Furthermore, it has been confirmed that this model is related not just to one particular target (as in targeted mutations; see 3.2), but to abnormalities in various neurotransmitter systems, including AVP (Landgraf et al., 2006; Wigger et al., 2004), serotonin (Keck et al., 2005), tachykinins (Sartori et al., 2005), CRF (Landgraf et al., 2006), oxytocin (Bosch et al., 2005) and possibly GABA/benzodiazepine (Kalisch et al., 2004). Disturbances in several neurotransmitter systems are also suspected in human psychopathology. On the basis of these findings and pharmacological validation studies it is suggested that HAB rodents can be considered as a relevant psychopathological model to study human anxiety disorders with face, construct and/or predictive validity (for review, see Landgraf and Wigger, 2003; Landgraf et al., 2006; Landgraf and Wigger, 2002). Recently, a similar selective breeding approach was performed in CD1 mice, resulting in very similar behavioral differences between HAB and LAB animals (Kromer et al., 2005).

3.2. Enhanced anxiety in mice with targeted mutation

Similarly to humans, fear and anxiety-like behaviors in rodents are polygenetically determined. Although it will not therefore be possible to target the 'gene for anxiety' in mutant mice, anxiety-related behavior can change considerably when one or more of the genes important in molecular pathways that mediate anxiety are modified. An important point to consider is that the strain, and therefore the genetic background, onto which a mutation is backcrossed can affect the detection of an anxiety-like phenotype in a mutant mouse (for review, see Belzung and Griebel, 2001; Cryan and Holmes, 2005). Belzung and Griebel (Belzung and Griebel, 2001) reported that already by 2001 thirty new strains of mice which display a phenotype consistent with increased anxiety had been generated by using gene targeting technology. Since 2001 this number has grown considerably. So far mainly knockout approaches and transgenic mice over-expressing certain genes have been described. Most of the approaches used conventional mutation of a gene, which has the potential for the induction of developmental alterations that may mask the normal function of the protein under study. More recently also 'conditional' mutations limiting

such adaptive changes have been exploited. Mice showing anxiogenic phenotype include knockouts of genes for GAD65, GABAA receptor γ2 subtype, 5-HT1A receptor, 5-HTT, α2A adrenoceptor, neurokinin 1 receptor, CRF2 receptor, neuropeptide Y, BDNF (conditional), glucocorticoid receptor (conditional knockout), mineral corticoid receptor, fyn protooncogene, MAS oncogene, as well as transgenic mice over-expressing genes, such as CRF (for review, see Belzung and Griebel, 2001; Finn et al., 2003; Lesch et al., 2003). These genetic animal models of anxiety provide an opportunity to study the relation and contribution of specific genes and their products to anxiety and possibly to emotional disorders. All these models are based on the deletion of a single gene, and since it is now clear that pathological anxiety involves the modulation of multiple genes, these approaches can only provide incomplete information.

3.3. Strains displaying spontaneously elevated anxiety-related behavior

Differences in anxiety-like behaviors across commonly used rodent strains and sublines have been reported extensively (e.g Bouwknecht and Paylor, 2002, for review see Finn et al., 2003). In rats, a number of strains displaying spontaneously elevated emotionality as compared with others, thought to model 'trait' anxiety, have been described, including Fischer-344 vs Wistar (e.g. Sudakov et al., 2001), fawn-Hooded vs Wistar or Sprague–Dawley rats (e.g. Kantor et al., 2000; Neophytou et al., 2000) and further examples (reviewed in Ramos and Mormede, 1998). In mice, in particular the BALB/c line has been proposed to model pathological anxiety, since it has shown higher levels of anxiety when compared with other strains, including C57BL/6, DBA/2, C3 H, CBA, and Swiss mice in various tasks (open field, light/dark choice test—for review, see Belzung and Griebel, 2001)—or when compared with NMRI mice in terms of grooming behavior (Kalueff and Tuohimaa, 2005, see, however, e.g. Avgustinovich et al., 2000). Interestingly, similarly to HAB animals, BALB/c mice have a high sensitivity to the anxiolytic action of benzodiazepines (for review, see Belzung and Griebel, 2001). Animals such as BALB/c mice are thought to exhibit increased anxiety because it is an enduring feature of a strain, probably involving multiple genetic and environmental factors.

3.4. Further approaches

3.4.1. Stress-based models of enduring anxiety

On the basis of the evidence that (chronic) stress exposures enhance the probability of developing psychopathology including anxiety disorders (Gutman and Nemeroff, 2003), it was investigated whether exposure of rodents to different stressors can induce enduring alteration in anxiety-related behavior. Indeed, it was shown in a number of studies that prenatal stress induces high anxiety in offspring as revealed by tests including the EPM, open field, and social interaction in rats (e.g. Vallee et al., 1997; Estanislau and Morato, 2005) and mice (Chung et al., 2005), while only a few studies have found no effect (Ordyan and Pivina, 2004). The early postnatal period and the bond between mother and infant seems particularly important in the development and shaping of normal emotional behavior (for recent reviews see Holmes et al., 2005; Pryce et al., 2005). Postnatal stress induced by maternal separation has been shown to produce permanent increases in anxiety-related behaviors when offsprings are tested as adults (for review see refs in Holmes). Higher anxiety later in life was observed for example on the EPM, open field or novelty suppressed feeding paradigm (Caldji et al., 2000; Francis et al., 2002; Wigger and Neumann, 1999), although results seem to be variable, since in addition, no effect or even reduction of anxiety has been found (Caldji et al., 2000; McIntosh et al., 1999). Also a rather variable picture emerges when adult rats or mice are chronically stressed by repeated restraint, chronic social or unpredictable stress, resulting in enhanced anxiety (Maslova et al., 2002), but also reduced anxiety (D'Aquila et al., 1994), or no change in anxiety-related behavior (D'Aquila et al., 1994; Harro et al., 1999). While adaptation mechanisms (see e.g. Girotti et al., 2006) are thought to be a likely source contributing to these discrepancies, it was proposed recenty that in addition to causing anxiety and coping deficits, chronic stress can elicit unspecific locomotor effects, interfering with the measurement of anxiety-related behavior (Strekalova et al., 2005). By selecting experimental conditions (e.g. low lighting) where this locomotor effect is absent, an increase in anxiety-related behavior was detected after chronic stress in mice (Strekalova et al., 2005). Interestingly, lasting increases in anxiety-related behavior have been reported even after brief exposures to stressors, including predator stress and administration of the benzodiazepine inverse agonist FG-7142 (e.g. Adamec, 2000; Adamec et al., 2004).

Several additional approaches to induce enhanced anxiety-like behavior that have not yet been studied widely include random mutagenesis (Finn et al., 2003) and RNAi (Thakker et al., 2005) techniques, as well as the possibility of inducing these changes via altered diet (e.g. Carrie et al., 2000; Singewald et al., 2004; Uchida et al., 2005).

4. Mapping of neuronal activation patterns in high-anxiety rodents

4.1. Altered neuronal activation processing revealed by challenge paradigms

In human symptom provocation imaging studies mainly *aversive* challenges, such as trauma-related sounds/pictures (in PTSD), viewing, for example, spider films in specific phobia, administration of anxiogenic drugs such as yohimbine (panic disorder, PTSD), aversive conditioning (panic disorder, social phobia), (fearful) face stimuli

(GAD, panic disorder, PTSD, social phobia), are used to reveal sites of dysfunctional neuronal processing in the brains of patients displaying pathological anxiety. A similar strategy—exposing individuals with excessive levels of anxiety to emotional challenges and revealing sites of dysfunctional neuronal processing—can also be applied in rodents. Challenges in these studies involve situations in which anxiety/stress is evoked, activating major parts of the fear/anxiety circuitry. Ideally, anxiety-related parameters including behavioral, autonomic (e.g. heart rate), and neuroendocrine (ACTH/corticosterone) responses can be evaluated and quantified at the same time. Selection of the challenge is critical, since stimuli that are too mild may activate parts of involved pathways insufficiently, while challenges that are too severe may induce ceiling effects in activation, obscuring putative differential activation. Furthermore, most regions comprising anxiety/fear circuitries are highly interconnected, and most of them will eventually get activated if salient anxiogenic stimuli are used (Gray and McNaugton, 2000). It is well known that conditioned and unconditioned fear/anxiety dissociate in neural circuitry (e.g. Walker et al., 2003; Wallace and Rosen, 2001); however, both types of stimuli can be used in humans (see above) and rodents in order to activate fear/anxiety-related brain areas. Important information concerning the brain areas that get activated in response to aversive challenges can be derived from extensive studies in unselected, 'normal' rodents. For example, the effects of a range of different stressors on brain activation are well known and show stressor specificity of Fos responses in different brain regions (for review, see Pacak and Palkovits, 2001). These findings are important for anxiety research, since a number of these stressors, including immobilization, have been shown to enhance anxiety-related behavior in rodents (e.g. Ebner et al., 2004) and additionally, pathways important in fear, anxiety and stress are known to exhibit considerable overlap. Using IEG mapping, several brain areas, including the medial prefrontal cortex (mPFC), bed nucleus of stria terminalis (BNST), lateral septum, specific hypothalamic regions, locus coeruleus (LC), and specific parts of the amygdala and PAG, have been reported to be activated in response to various aversive, fear/anxiety-provoking challenges, including exposure to the EPM, light/dark test, conditioned fear, airpuff or administration of anxiogenic drugs (e.g. Beck and Fibiger, 1995a; Campeau et al., 1997; Duncan et al., 1996a; Kabbaj and Akil, 2001; Knapp et al., 1998; Kovacs, 1998; Palmer and Printz, 1999a, b; Silveira et al., 1993; Singewald et al., 2003; Singewald and Sharp, 2000). It appears that there is a specific pattern of Fos induction in the brain that may be a "fingerprint" of anxiogenic stimuli that is clearly different to that evoked by stimuli unrelated to anxiety, although one must consider that each individual area, such as, for example, the amygdala, can indeed be activated by a wide range of stimuli and drugs not associated with fear or anxiety (e.g. Phan et al., 2002). In fact, anxiolytic drugs activate subregions of this nucleus, underlining the requirement of mapping techniques with high spatial resolution (see also Section 4.2). Many of these identified brain areas have also been found to be activated (indicated by enhanced CBF) by fear/anxiety-related stimuli in 'healthy' human volunteers (e.g. Fredrikson et al., 1995; Ploghaus et al., 1999) and have been implicated in fear/anxiety mechanisms based on studies including stimulation and ablation work (for review, see Millan, 2003). Taken together, this evidence underlines the idea of integrated neuronal circuits that are common targets for stimuli that are linked to anxiety. Dysfunction in parts of these circuits is likely to lead to dysfunction in the processing of anxiety and ultimately to, for example, exaggerated fearful reactions to aversive stimuli, which is a characteristic of particular anxiety disorders (for review, see Marshall et al., 1999). However, it is not well understood which changes in which parts of proposed anxiety circuitries predispose individuals to be hyperanxious.

Building on these studies in 'normal' rodents, approaches to identify neuronal circuits showing dysfunctional processing in animal models of 'pathological' anxiety are currently evolving by exposing high-anxiety rodents (Section 3) to aversive situations. So far only a relatively small number of different aversive stimuli in some diverse 'high-anxiety rodent models' have been studied (outlined in Sections 4.1.1–4.1.3). The selection of the aversive stimulus should also take into consideration the fact that it has been demonstrated that different sets of brain structures are activated in rats depending on the behavioral performance (inhibitory avoidance vs escape) to aversive situations (Silveira et al., 2001), underlining the suggestion that different anxiety states in animals may recruit and involve different brain regions. In order to increase the possibility of detecting disturbances in neuronal activation that are important in various aspects of anxiety, different aversive stimuli should ideally be investigated, ensuring that most of the affected pathways indeed get activated, which is a prerequisite for unraveling potential differences in the neuronal activation pattern.

4.1.1. Functional mapping in selectively bred rodents

In the HAB/LAB lines, selectively bred for extremes in anxiety-related behavior (see 3.1), differences in cerebral neuronal processing in response to a number of different aversive stimuli or anxiety-provoking situations have been revealed by using mainly Fos expression as a marker of neuronal activation. These stimuli so far include exposure to the open arm of an EPM, open field (Salome et al., 2004), social defeat (Frank et al., 2006), airjet and administration of the anxiogenic drug FG-7142 (Salchner et al., 2006) in rats, as well as, in a preliminary study, open arm exposure in mice (Muigg et al., 2005). Unselected, Normal Anxiety Behavior (NAB) animals, which display intermediate anxiety-related behavior in most tests (see, e.g. Kromer et al., 2005) were also studied in select mapping experiments. Unfortunately, in other selectively bred rat models apart from HAB and LAB

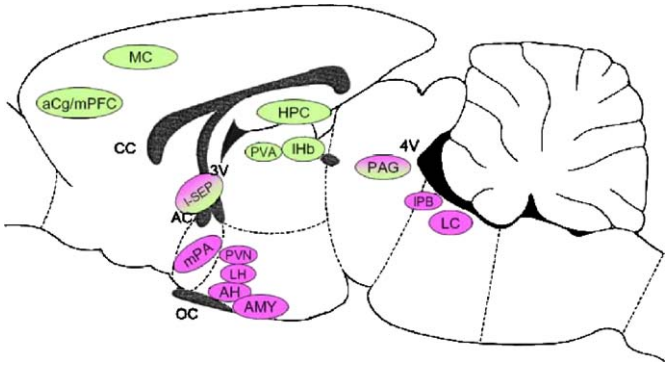

Fig. 1. This figure depicts the sites of differential neuronal activation to five different challenges in high-anxiety HAB rats vs. low-anxiety LAB rats as assessed by Fos mapping. Red: hyperactivation, green hypoactivation in HABs vs. LABs. Challenges used were exposure to the open arm of an elevated plus maze, open field, social defeat, air jet and administration of the anxiogenic drug FG-7142. For further details see text. MC, motor cortex; aCg/mPFC, anterior cingulate/medial prefrontal cortex; l-SEP, lateral septum; mPA, medial preoptic area; PVN, paraventricular hypothalamic nucleus; AH, anterior hypothalamic area; PVA, paraventricular thalamic nucleus; LH, lateral hypothalamic area; HP, hippocampus; AMY, amygdala; lHb, lateral habenular nucleus; dlPAG, dorsolateral periaqueductal gray; lPB, lateral parabrachial nucleus; LC, locus coeruleus. 3V, 3rd ventricle; 4V, 4th ventricle, AC anterior comissure, OC optic chiasm.

Table 2
Differences in challenge-induced Fos expression between HAB and LAB rats

Brain regions	Challenges				
	OA	OF	SD	AJ	FG
Cortex					
Cingulate cortex	↓	↓	↓		↓
Prelimbic cortex					↓
Infralimbic cortex					↓
M1/M2		↓	↓		
Hippocampal formation					
Dendate gyrus	↓				
CA3	↓				
Amygdala					
Central nucleus of the amygdala			↑		
Medial nucleus of the amygdala			↑		
Septum (lateral)					
Ventral	↑				
Intermediate			↓		
Hypothalamus					
Paraventricular hypothalamic nucleus	↑	↑	↑ (pv)		
Anterior hypothalamic area	↑	↑		↑	↑
Lateral hypothalamic area	↑	↑			
Medial preoptic area	↑	↑	↑	↑	↑
Thalamus					
Paraventricular thalamic nucleus		↓	↓		
Lateral habenular nucleus		↓			
Midbrain/pons					
Periaqueductal gray, rostral					
Dorsolateral				↑	
Dorsomedial			↓		
Lateral parabrachial nucleus	nd	nd	nd		↑
Locus coeruleus	nd		nd	↑	↑

↑ indicates enhanced Fos response in HABs, ↓ indicates attenuated Fos response in HABs as compared to LABs. Only brain areas are given where differential activation patterns between the lines were observed. Note that these areas constitute only a fraction of the investigated areas (for details see text).
Abbreviations: AJ, air jet exposure; FG, administration of the anxiogenic drug FG-7142; nd, not determined; OA, open arm exposure; OF, open field exposure; pv periventricular zone, SD, exposure to the social defeat paradigm.

(Table 1), mapping of neuronal activation patterns has been used only on a few occasions, for example, in studying the effect of intracerebral CRF infusion (Wiersma et al., 1998). This lack of information excludes the possibility of comparing altered neuronal activation patterns across the different models of diverse anxiety-related behavior. In HAB and LAB rats, challenge-induced neuronal activation as compared to non-challenged controls was observed in widespread brain regions, including major parts of the fear/anxiety circuitry. Activated neurons and brain areas generally corresponded well with published findings in unselected animals. In a fraction of activated regions the evoked brain activity pattern differed between HAB and LAB rats, revealing both hyper- and hypoactivation of specific neuronal populations in HABs as compared with LABs (Fig. 1). Sites of differential Fos response between HABs and LABs in response to these challenges were particularly identified in limbic, hypothalamic and hindbrain areas, suggesting that disturbance in these systems and associated circuitries contribute to the diverse behavioral and neuroendocrinological responses between the lines. The identification of neuronal populations showing differential activation depends in part on the applied emotional challenge (Table 2), indicating that specific facets of elicited aversion, fear or anxiety preferentially engage particular parts of the fear/anxiety circuitry (see below).

Similarly, task dependence has also been proposed in human imaging studies (Bremner, 2004).

4.1.1.1. Limbic system. The mPFC is part of the limbic system and provides important inhibitory inputs to the amygdala. It comprises several related areas, including the anterior cingulate, prelimbic and infralimbic cortex. In HABs, hyporesponsive neurons to different challenges (open arm, open field, social defeat and FG-7142) were found in the anterior cingulate cortex (Frank et al., 2006; Salchner et al., 2006; Salome et al., 2004). This region in the dorsal mPFC which is considered as an interface between emotional and cognitive functions (Allman et al., 2001) has been implicated in anxiety mechanisms (for review, see Goddard and Charney, 1997; Gray and McNaughton, 2000). Attenuated activation in response to FG-7142 was also observed in the ventral mPFC cortex (infralimbic and prelimbic), which projects to the amygdala, septum, medial

parts of the preoptic and hypothalamic areas (for review, see Heidbreder and Groenewegen, 2003), all of these being neuronal populations that were hyperactivated in HABs (see below). Although there is some debate about the homology of medial prefrontal cortical areas in rodents and humans (e.g. Drevets, 2001), it is interesting that hypofunction of the cingulate cortex was reported in specific anxiety disorders such as PTSD (Bremner, 2004; Cannistraro and Rauch, 2003). Accordingly, the hyporesponsiveness of the anterior cingulate/mPFC in response to stressful challenges may contribute to the hyper-anxious phenotype of HABs, since it was observed in unselected rodents that lesions of the mPFC comprising the anterior cingulate cortex and dorsal prelimbic cortex are associated with suboptimal responses to stress and anxiogenic-like stimuli (Jinks and McGregor, 1997; Morgan and LeDoux, 1995; Vouimba et al., 2000), reviewed in Sullivan and Gratton (2002, see, however, e.g. Shah and Treit, 2003). Lesions of the anterior cingulate and prelimbic divisions of the mPFC have also been shown to enhance ACTH and corticosterone secretion, as well as paraventricular hypothalamic nucleus (PVN) c-fos expression in response to specific stressors (Herman et al., 2005). Interestingly, in a fMRI study, the most pronounced difference between HAB and LAB animals was found in the anterior cingulate/mPFC, with a significantly lower responsiveness of HAB than LAB rats to the depressant action of diazepam (Kalisch et al., 2004), supporting the finding that high-anxiety HABs and low-anxiety LABs differ in neuronal processing within the prefrontal cortex. Recent preliminary findings showing that HAB rats display greatly delayed extinction of conditioned fear compared with LABs or NABs (Hetzenauer et al., 2005) may be consistent with disturbed neuronal processing in the mPFC (for review, see Maren, 2005) and other limbic areas (see below).

The amygdala which contains a number of different subnuclei is an important limbic brain area involved in the processing, expression and integration of anxiety-related emotion and is thought to play a key role in assigning emotional significance to specific sensory input (e.g. Davidson et al., 2002; Anand and Shekhar, 2003). Within this brain region, enhanced Fos response in HABs as compared with LABs was revealed in the social-defeat paradigm specifically in the central and medial amygdala (Frank et al., 2006). HABs displayed greater ultrasonic vocalization and freezing responses than in LABs, suggesting an enhanced fear/anxiety state in HABs following social defeat. The central amygdala is thought to be important in the mediation of fear and anxiety responses (e.g. Kalin et al., 2004), but this does not seem to apply to all anxiety states equally (e.g. Li et al., 2004). Both the central and the medial amygdala are involved in the regulation of the hypothalamic–pituitary–adrenal (HPA) axis (Dayas et al., 1999; Herman et al., 2005; Ma and Morilak, 2005), thought to mediate activation of the HPA axis (Herman et al., 2005). Increasing evidence indicates that the medial amygdala is also of importance for specific fear and anxiety mechanisms, including unconditioned fear/anxiety towards predator odor and stress-induced anxiety (Blanchard et al., 2005; Ebner et al., 2004; Li et al., 2004). In a recent preliminary fear-conditioning study (Hetzenauer et al., 2005) differential Fos expression between HABs and LABs was observed not only in the central and medial amygdala, but also in the lateral amygdala (Muigg and Singewald, unpublished), suggesting that pathways involving parts of the amygdala display differential excitability in high- and low-anxiety rats, respectively. Increased amygdala responsivity appears to be subject to the task type, since it was not revealed in open arm or open field exposure (Salome et al., 2004). Given the functional connectivity and interaction between prefrontal/cingulate cortex and amygdala in animals (Maren and Quirk, 2004; Sotres-Bayon et al., 2004) and humans (Pezawas et al., 2005), the observed hyperactivation in the amygdala along with hypoactivation in the cingulate/mPFC of HABs may be directly correlated. This issue deserves further investigation given the evidence that changes in the functional integrity of this circuit which seems also disturbed after chronic stress (Correll et al., 2005) predicts variation in temperamental anxiety (Pezawas et al., 2005).

The septum and hippocampus, in which differential Fos responses between HABs and LABs were observed following open arm exposure (Salome et al., 2004) and social defeat (Frank et al., 2006), are integral parts of the septo-hippocampal system and have been implicated in anxiety and memory mechanisms (for review, see Bannerman et al., 2004; Gray and McNaugton, 2000). For example, septal lesions have been shown to exaggerate defense reactions (Blanchard et al., 1979) and accordingly septal activation can suppress responses to adverse emotional states (Yadin et al., 1993). However, there is evidence that substrates for both anxiety-relieving and anxiety-provoking mechanisms exist within the lateral septum (Degroot et al., 2001; Kask et al., 2002; Menard and Treit, 1999, for review, see Sheehan et al., 2004). These different subregions of the lateral septum mediating in part opposing affective functions are known to differ in their neurochemical make-up and the interconnections with other brain areas. For example, it was proposed that the ''ventral lateral septum'' promotes fear and passive coping responses such as freezing, whereas the ''rostral lateral septum'', strongly interconnected with medial hypothalamic sites, inhibits defensiveness. The posterodorsally located ''caudal lateral septum'' with its connections to locomotor-activating regions in the lateral hypothalamus is thought to modulate active coping responses (for review, see Sheehan et al., 2004). The detected hyperactivation in the ventral lateral septum along with hypoactivation in the intermediate part (Table 2) is in line with such proposals, underlining the importance in future studies of identifying the neurochemical phenotype of the differentially activated neurons within the septum.

4.1.1.2. Hypothalamus/thalamus. In distinct regions of the medial hypothalamus, specifically the anterior hypothalamic area and the medial preoptic area (Frank et al., 2006; Salome et al., 2004), neurons were identified showing hyperactivation in HABs in response to 4 and all 5 of the challenge paradigms, respectively. Although enhanced Fos expression in the anterior hypothalamic area and the medial preoptic area was noted in animal tests of anxiety (Campeau et al., 1997; Duncan et al., 1996a; Emmert and Herman, 1999; Silveira et al., 1993) and these areas are known to be involved in the HPA axis response (Feldman et al., 1990), their relation to anxiety mechanisms is not entirely clear. Both regions are suggested to be integral parts of a hypothalamic circuit subserving defensive responses which represent a behavioral response to fear (Canteras, 2002; Canteras et al., 1997; Dielenberg et al., 2001; Sewards and Sewards, 2002). As extreme defensive responses occur in dispositionally fearful humans who have an increased risk to develop psychopathology (Bakshi et al., 2000), the increased Fos expression in these regions may underlie the fearful endophenotype of the HAB rats, independent of the stimuli used. In the light of these common findings in the hypothalamus, it is worth considering whether test-induced differences in locomotor behavior may possibly contribute to them. However, this does not seem to be the case, since quite different locomotor-associated responses in HABs vs LABs were observed, including decrease (e.g. open field), increase (e.g. airjet), or no difference (e.g. open arm), despite common Fos hyperresponse in both hypothalamic areas. Furthermore, while hypoactivation in the motor cortex (M1, M2) was observed in HABs after open field and social defeat, which seems consistent with indices of lower locomotion and higher freezing, respectively, in these tests, no differences between the two lines were observed in response to any other challenge (open arm, airjet, FG-7142) in these areas. In particular, the higher number of escape jumps after airjet in HABs was not associated with an enhanced Fos response in these motor areas, suggesting that the difference in motor behavior in this test was too low to be picked up by Fos mapping.

Further neuronal populations in the hypothalamus showing challenge-induced hyperactivation in HABs were found in the lateral hypothalamic area, which is thought to be involved, for example, in the autonomic expressions of fear and anxiety (Charney, 1998), as well as in the PVN. These neuronal populations were found in the parvocellular and magnocellular part of the PVN (Salome et al., 2004), as well as in a (ventral) periventricular zone of the PVN (Frank et al., 2006). Neurons in the PVN are particulary important in the integration of stress reactions and have been shown to be activated in response to environmentally and pharmacologically induced anxiety (Emmert and Herman, 1999; Kabbaj and Akil, 2001; Mulders and Chan, 1995; Nagahara and Handa, 1997; Silveira et al., 1993; Singewald et al., 2003). Interestingly, c-fos antisense-treated socially defeated rats displaying decreased Fos expression in the PVN displayed a reduction in anxiety on the EPM, compared with vehicle- or sense-treated rats (Lu et al., 2000), further supporting the association of enhanced Fos expression in the PVN with anxiety. The finding of hyperresponsive neurons in the PVN of HAB rats is consistent with neuroendocrine data indicating a hyper-reactive HPA axis in HABs (for review, see Landgraf and Wigger, 2002), and neurochemical data showing hypothalamic hyperexpression of AVP (Wigger et al., 2004) and CRF (Landgraf et al., 2006) in HABs.

In the thalamus, a blunted Fos response in HABs was noted in the lateral habenula and in particular in the paraventricular thalamic nucleus in response to open field exposure and social defeat (Salchner et al., 2006). The significance of this finding is not clear at the moment but deserves further evaluation, since the paraventricular thalamic nucleus with connections to the mPFC and the amygdala is part of the viscerolimbic midline nuclei involved in arousal, amongst other functions (Van der Werf et al., 2002), and hypoactivation of this region has also been suggested, for example in PTSD patients as compared with healthy volunteers (Bremner, 2004).

4.1.1.3. PAG and brain stem. In another main site for executive mechanisms of the defense reaction (for review, see Bernard and Bandler, 1998; Lovick, 1996), the dorsolateral periaqueductal gray (PAG), an increased Fos response in HABs was noted following administration of FG-7241 and airjet exposure, which elicited escape responses that were more pronounced in HABs (Salchner et al., 2006). In accordance with these findings, previous studies have shown that stimuli inducing escape behavior activate Fos expression in this brain region (Lamprea et al., 2002; Neophytou et al., 2000; Silveira et al., 2001). Moreover, electrical or chemical stimulation of the dorsal PAG has been shown to elicit an explosive flight reaction that resembles a panic attack which is associated with intense anxiety (Jenck et al., 1995). After social defeat, a lower number of Fos positive neurons in HABs was observed in a more medial aspect of the PAG. It will be important to identify the chemical phenotype of the affected neurons to draw conclusions on this pattern of differential activation in the PAG.

Brainstem areas were unfortunately not investigated following open arm exposure or social defeat. However, similar to the dorsolateral PAG, hyperactivation in the LC of HABs was observed in response to the same two stimuli, anxiogenic drug administration and airjet exposure (Salchner et al., 2006). This difference was not found following open field exposure, which, however, does not necessarily reveal differential neuronal responding, since it does not activate LC neurons sufficiently either in HABs or LABs (Salchner and Singewald, unpublished). This finding underlines the notion that anxiety, as defined in a given animal test, differs from that generated in other tests representing distinct endophenotypes (Andreatini et al., 2001; Battaglia and Ogliari, 2005; Pratt, 1992). Thus, tests

based on escape behavior in response to an unconditioned threat stimulus are thought to model the persistent 'fight/flight' behavior exhibited by patients suffering from extreme anxiety states such as panic disorder, while models based on exploratory behavior such as the open field are thought to predominantly model certain aspects of generalized anxiety (Andreatini et al., 2001; Blanchard et al., 2001; Bourin, 1997; King, 1999; Shekhar et al., 2001). Since administration of FG-7142 which is anxiogenic in rodents (see refs in Singewald et al., 2003) has also been shown to elicit severe anxiety states in humans, with symptoms resembling those of a panic attack (Dorow et al., 1983; Horowski and Dorrow, 2002) it might be speculated that hyperresponsiveness of LC neurons is predominantly a critical factor in severe anxiety states. Accordingly, inhibition of enhanced LC/noradrenergic responses has been proposed to underlie attenuated stress-induced anxiety responses (Ma and Morilak, 2005).

Taken together, these findings suggest that the differential activation pattern in HABs and LABs is mainly anxiety related, while other possible effects of the applied challenges, such as changes in locomotor parameters, do not seem to contribute to a significant extent. Since blunted Fos responses in HABs were also found, it is clear that neuronal hyperexcitability is not a general feature of neuronal processing in HABs. Hyperexcitability of neuronal populations in HABs was indeed detected, but it appeared to be confined to distinct pathways implicated in fear/anxiety mechanisms. In preliminary experiments in the recently developed respective mouse model (Kromer et al., 2005), similar differences in challenge-induced Fos activation patterns between HAB and LAB lines were observed. Fos and behavioral responses in NAB mice in these experiments were intermediate or more similar to responses found in LAB mice (Muigg et al., 2005), suggesting that neuronal processing in HAB animals also differs to that of unselected animals displaying 'normal' anxiety-related behavior. The 'abnormal' neuronal processing in hyperanxious HABs, however, may either indicate exaggerated activation of pathways mediating anxiety or represent dysfunctional adaptive responses which normally serve to suppress anxiety. Part of the altered activation pattern in HABs may even be the expression of (insufficient) counter-regulatory mechanisms aimed at inhibiting the enhanced anxiety response in this line. Hence, at this point it cannot be decided which of the identified differences in neuronal activation is truly maladaptive/adaptive and should be counteracted/strengthened to elicit anxiolytic effects. Further studies, for example employing treatment with different anxiolytic drugs (see below) and/or Fos antisense approaches, are necessary to further clarify this issue.

4.1.2. *Functional mapping in mice with targeted mutation*

A number of functional mapping studies have been performed in genetically modified mice showing enhanced anxiety. However, in most cases only a small number of brain areas were investigated, rather than applying global mapping of large parts of the brain. For example, in female oxytocin KO mice, which display an anxiogenic phenotype in the EPM and enhanced stress-induced hyperthermia, only the medial amygdala was investigated in one study (Amico et al., 2004). OXT−/− mice showed increased Fos expression in the MeA, compared with wildtype mice following EPM exposure. In a second study, no differences in Fos response between the genotypes were observed after shaker stress in the central amygdala, BNST, medial preoptic area or paraventricluar area of the thalamus, while a reduced response was found in the medial amygdala of OXT−/− (Mantella et al., 2004). Linden et al. (2003) observed that the increased anxiety of metabotropic glutamate 8 receptor knockout mice in the EPM was associated with higher Fos expression in the centromedial nucleus of the thalamus following this test. However, 5 min of EPM exposure in these experiments did not significantly activate anxiety-related brain areas such as amygdala, mPFC, or LC, rendering investigation of differential activation between the two genotypes largely impossible. This was likely due to the conditions the authors had to choose—dim lighting conditions and prehandling of the mice—which obviously reduced the stressfulness of the aversive situation. This was necessary since only under these conditions a difference in time spent in the open arm between the genotypes was detectable. Using an auditory fear-conditioning paradigm, Kubota et al. (2004) observed that the enhanced freezing response during 5 min tone (CS) presentation in Fyn-tyrosine kinase knockout mice as compared with heterocygotes is associated with enhanced Fos response in parts of the amygdala (including medial, basomedial, central and cortical nuclei), the PVN, lateral hypothalamus and parts of the PAG, while attenuated Fos response was noted in basolateral and lateral nuclei of the amygdala. The authors concluded that the enhanced excitability of the origins and targets of the amygdalo-hypothalamic and amygdalo-midbrain projection systems elicits enhanced emotional reactions and is related to the increased fearfulness of Fyn-tyrosine knockout mice. Conditioned fear was also studied in heterozygous mice deficient of the sodium pump $\alpha 2$ subunit (Atp1α2), which show enhanced anxiety-related behavior in the EPM, light/dark test and open field test (Ikeda et al., 2003). In a contextual fear-conditioning paradigm these mice displayed exaggerated freezing behavior and enhanced Fos response in the amygdala and piriform cortex (Ikeda et al., 2003). Although, unfortunately, no other brain areas were investigated, the authors concluded that 'neuronal hyperactivity in these regions may be the cause of the increased fear/anxiety behavior' of these mutant mice. Finally, male CRH2 receptor KO mice, which show enhanced anxiety-related behavior in the EPM, light/dark emergence task and the open field test, were studied (Kishimoto et al., 2000). Following 5 min exposure to the EPM no difference in the Fos immunoreactivity (1 h post EPM exposure) was noted. Unfortunately, no details concerning brain areas investigated and the extent of

possible neuronal activation by EPM exposure using this rather short post-stimulus interval were given. It was, however, noted that phosphorylated CREB immunoreactivity was attenuated in the cingulate cortex, the BNST, the ventromedial hypothalamic nucleus and the basolateral amygdala, which was associated with the lack of the CRH2 receptor (Kishimoto et al., 2000).

Although it is doubtful whether a single targeted mutation, despite producing enhanced anxiety-related behavior in anxiety tests, is a useful model to study pathological anxiety (Belzung and Griebel, 2001), these studies provide interesting information concerning the site of action and function of the targeted gene in the processing of aversive stimuli, such as, for example, the involvement of Fyn-tyrosine kinase in the expression of conditioned fear. A major shortcoming at the moment is the lack of *global* mapping studies involving such rodents with targeted mutations.

4.1.3. Functional mapping in independent strains displaying differential anxiety-related behavior

Differential processing of neuronal activity in mouse strains differing in anxiety-related behavior has not been investigated systematically so far. As an example, in a study investigating the Fos response in striatal areas, BALB/c mice were compared with C57BL/6 mice in response to amphetamine and a D1 receptor agonist, but unfortunately no aversive emotional stimuli were studied (Conversi et al., 2004). In a preliminary study, it was observed that the Fos response to open arm exposure in BALB/c mice differs from that in C57BL/6 in brain areas including the basolateral and central amygdala, the PVN, the medial preoptic area, the hippocampus, and parts of the PAG (Hetzenauer, Sartori, and Singewald, unpublished). Examples using this approach in rats include comparison of Fischer and Lewis rats, revealing differential Fos response to water avoidance stress in the PVN (Million et al., 2000), or hooded Lister vs Wistar rats which exhibit distinct defensive behavior and differential Fos response to aversive ultrasound in different parts of the PAG (Neophytou et al., 2000). WKY rats were compared with Sprague–Dawley rats to address the possible neurobiological mechanisms underlying dysregulation of the stress response in WKY rats. Immobilization stress-induced Fos expression was lower in WKY rats compared to Sprague–Dawley rats in the LC and in the medial amygdala, whereas there were no strain differences in other regions examined, including the BNST, the central amygdala and the PVN (Ma and Morilak, 2004). This attenuated neuronal activation was associated with a previously noted reduced anxiety-like behavioral reactivity in WKY rats. These data seem comparable to observations in LABs that also show (compared to HABs) reduced anxiety-like behavior and attenuated stress-induced neuronal activation in the medial amygdala and the LC, among other areas. One possible disadvantage in using strains with different genetic backgrounds that differ in anxiety-related behavior is that the Fos expression pattern even under 'home cage conditions' can differ considerably (Neophytou et al., 2000), making direct comparisons of challenge-induced Fos responses as well as their undoubted relation to fear and anxiety more difficult.

4.1.4. Functional mapping in further models

In stress-based models (see also 3.4), a number of functional mapping studies were conducted which up to date mainly focused on very few specific brain areas and rarely investigated anxiety-related behavior in the same study. Enhanced prefrontal Fos response to restraint was found in prenatally stressed rats (Rosene et al., 2004). In adult rats that had experienced postnatal maternal deprivation enhanced Fos response to mild stressors was noted in the PVN (Levine, 2002; Smith et al., 1997), while postnatal handling, which is thought to exert anxiolytic effects (e.g. Vallee et al., 1997), attenuated stress-induced Fos response in the PVN, the central amygdala, the BNST, the hippocampus, the posterior cingulate cortex and the piriform cortex (Abraham and Kovacs, 2000).

In addition to selective breeding, acute selection, for example on the EPM, was also performed to investigate individual differences in reactivity (e.g. coping style) to anxiogenic/stressful situations, as well as underlying IEG expression patterns. While it is not clear at the moment if these differences in anxiety-related behavior are enduring and how these models relate to pathological anxiety, interesting differences in IEG mapping were revealed. For example, rats that exhibited low reactivity to novelty and high anxiety-like behavior showed attenuated Fos response to an anxiogenic stressor (EPM exposure) in the cingulate cortex, the orbital cortex, the dorsal striatum and the PVN, while enhanced activity was noted in the CA1 region of the hippocampus as compared with rats that were hyperresponsive to novelty (novelty seeking) and exhibited low anxiety-related behavior (Kabbaj and Akil, 2001). On the other hand, rats showing high and low grooming on the EPM (Koya et al., 2005) did not differ in IEG expression (egr1, egr2 or c-fos) in the three brain areas investigated (the mPFC, the nucleus accumbens shell and the ventral tegmental area) following exposure to the EPM test. The authors suspected that the limited anatomical resolution of the method applied (tissue dissection and real time quantitative PCR) may explain the failure to detect differences in IEG expression. It should be noted that no differences in classical anxiety parameters such as open arm entries or time on open arms were observed between the lines.

4.2. Modulation of altered activation patterns by therapeutic drug treatment

It has been suggested that functional mapping studies and in particular Fos expression profiling are suited to gaining information on brain targets important for the efficacy of existing and experimental anxiolytic drugs and

can aid the therapeutic classification of psychoactive drugs including anxiolytics (for review, see Sumner et al., 2004). A number of studies investigating anxiety reduction by drugs (e.g. Beck and Fibiger, 1995a; McGregor et al., 2004), environmental manipulations (e.g. Abraham and Kovacs, 2000), or targeted mutation (e.g. Singewald et al., 2005) provide evidence for a relation of altered Fos response in specific brain areas to reduced behavioral, autonomic and neuroendocrine anxiety responses. In these studies 'inescapable' situations were chosen as challenges (e.g. conditioned fear, restraint, exposure to the open arm of an EPM), while challenges involving situations in which rodents have a choice to respond (e.g. entering enclosed arms in the EPM) seem to be less appropriate for such investigations (Jinks and McGregor, 1997; Kishimoto et al., 2000). A very important issue in these experiments is a high spatial resolution of the mapping technique applied and thus the possibility to evaluate neuronal activity changes even in subregions of brain nuclei. While anxiolytic drugs such as benzodiazepines enhance (basal) Fos expression in select areas, including (specific subregions of) the central amygdala and the BNST (e.g. Hitzemann and Hitzemann, 1999), attenuation of challenge-induced Fos induction by benzodiazepines was noted in various cortical brain areas, as well as subcortical areas, including the lateral septum, the BNST, the nucleus accumbens, the hippocampus, the hypothalamus (e.g. lateral, dorsomedial, preoptic area) and the thalamus (paraventricular, lateral habenula) (Beck and Fibiger, 1995a; de Medeiros et al., 2005; McGregor et al., 2004; Titze-de-Almeida et al., 1994). Furthermore, also other drugs used to treat anxiety such as buspirone (Wislowska-Stanek et al., 2005) or different antidepressants (Beck and Fibiger, 1995b; de Medeiros et al., 2005; Duncan et al., 1993, 1996b; Lino-de-Oliveira et al., 2001; Morinobu et al., 1995), were shown to modulate challenge-evoked neuronal activity patterns in emotion-related brain areas. Unfortunately, such studies are scarce in rodent models of enhanced (pathological) anxiety:

4.2.1. *Selectively bred rats*

Chronic SSRI (paroxetine) treatment via the drinking water attenuated challenge-induced Fos hyperexpression in HAB rats in the PVN and a number of additional brain areas, including the amygdala, the lateral septum, the PAG and the LC, while no paroxetine effect was noted for example in the paraventricular thalamic nucleus, lateral habenular nucleus or motor cortex (Muigg et al., 2004). It should be mentioned that while this treatment attenuated the enhanced depression-like behavior in the forced swim test used here as the challenge, a putative anxiolytic effect was not evaluated in this study. In a preliminary experiment investigating only select brain areas, pretreatment of HAB rats with diazepam was shown to attenuate hyperexpression of Fos in the PVN and LH following exposure to the open arm of an EPM (Muigg and Singewald, unpublished). The diazepam treatment was associated with a clear anxiolytic-like effect, indicated by enhanced risk assessment (head dips) and enhanced time spent on the presumably more aversive distal end of the open arm, which is in good accordance with the anxiolytic effect of this drug in HABs on the EPM test (Liebsch et al., 1998).

4.2.2. *Mutant mice and further models*

Differential Fos response in genetically modified animals showing an anxiolytic-like phenotype as compared with respective wildtypes has been observed in a preliminary study. Conditional CRH1 KO mice, showing an anxiolytic-like phenotype in different tests, including the EPM (Muller et al., 2003), displayed attenuated Fos response to open arm exposure in brain areas such as the amygdala, the hypothalamus, the lateral septum and the prelimbic cortex (Singewald et al., 2005), suggesting that modulation of neuronal activation in these areas may be associated with attenuation of anxiety-like behavior.

Taken together, the experimental evidence so far points to a number of specific brain areas, including the amygdala, the hypothalamus and the lateral septum, where neuronal hyperexcitability to challenges in high-anxiety rodents is reversed by different drugs known to exert anxiolytic efficacy in rodents and humans. More extensive investigation using different rodent models of enhanced anxiety, as well as different challenging paradigms and also different drugs, is warranted to further reveal which of the changed activity patterns in high-anxiety individuals should be targeted in order to attenuate anxiety. Furthermore, combining functional mapping studies with appropriate neurochemical studies should provide information concerning the different targets and pathways by which this can be accomplished.

5. Integration of animal and human findings

An important issue is the potential relationship of the rodent findings reviewed above to human psychopathology. Although generally extreme caution is advisable in extrapolating findings in rodents to humans, considerable evidence indicates that basic mechanisms mediating/modulating anxiety including neurotransmitters and the neurocircuitries involved are considerably conserved across species. Indeed, a number of conclusions drawn from animal fear/anxiety work have been confirmed in human studies (e.g. Gorman et al., 2000; Vermetten and Bremner, 2002a, b). Hence, it is likely that the neuronal populations/brain areas showing aberrant challenge-induced neuronal activation in high-anxiety rodents may also be affected in humans with pathologically enhanced anxiety (as revealed in challenge paradigms). Indeed, recent symptom provocation studies in humans with anxiety disorders such as PTSD, panic disorder, social phobia, and GAD have shown aberrant neuronal activation in a number of brain areas, including many of those identified in the rodent studies (for review, see Bremner, 2004; Cannistraro and

Rauch, 2003; Talbot, 2004). Interestingly, there is also considerable correspondence between the rodent data (see Section 4) and human findings for example in PTSD (for review, see Bremner, 2004), concerning the direction of altered activation in response to challenges (i.e. relative hyperactivation, e.g. in the amygdala, and hypoactivation in the anterior cingulate/mPFC, the hippocampus and the thalamus). Although different brain areas have been shown to be affected in different anxiety disorders, supporting neuroanatomic distinctions between these disorders, it is interesting that specific brain areas seem to show similar aberration in neuronal activation across many anxiety disorders, suggesting the likelihood that commonly affected neurocircuitry elements are a basis for the symptom overlap in the anxiety disorders (for review, see Bremner, 2004; Cannistraro and Rauch, 2003; Kent and Rauch, 2003). The amygdala is one such example, showing hyperexcitability in symptom provocation studies in PTSD, social phobia (for review see Bremner, 2004; Cannistraro and Rauch, 2003) and in one recent study also in GAD (Thomas et al., 2001). In panic disorder, evidence for both hyperactivation and hypoactivation has been provided (Bremner, 2004). Hypoactivation of anterior cingulate in response to different challenges was observed in most studies in PTSD, while in other anxiety disorders results were more inconsistent, also revealing hyperactivation, in particular in obsessive–compulsive disorder (Bremner, 2004; Cannistraro and Rauch, 2003). Unfortunately, human symptom provocation imaging studies including areas identified as being affected in rodents, such as the hypothalamus, the septum, the PAG and the LC, are scarce, most likely due to the size of these areas and/or technical problems in reliably scanning brain areas in laminar structures such as the brain stem. However, the available data seem to be in agreement with the rodent findings. In the hypothalamus, hyperactivation was revealed in panic disorder, the only investigated anxiety disorder for that part of the brain so far. Similarly, hyperactivation of the 'PAG/midbrain' was observed in panic disorder and PTSD (Boshuisen et al., 2002; Rauch et al., 1997) and one study has shown hyperactivation in the 'pons' in social phobia (Lorberbaum et al., 2004).

The interaction of therapeutic drug treatment and clinical improvement with symptom provocation-induced brain activation has not yet been widely studied in human neuroimaging studies. In two PET studies, chronic treatment of social phobia patients with the SSRI citalopram (Furmark et al., 2002) or the NK1 receptor antagonist GR205171 (Furmark et al., 2005) led to anxiety reduction and reduced the heightened neural response to public-speaking challenge in the medial temporal lobe, including the amygdala, as well as the perirhinal, entorhinal, and parahippocampal cortices. A similar effect on neuronal responses was also noted after cognitive behavioral therapy (Furmark et al., 2005). These findings led the authors to suggest that the downregulation of medial temporal lobe neuronal activity is an important mechanism in the alleviation of social anxiety (Furmark et al., 2005). In an fMRI study in patients with GAD, reduced BOLD responses to worry and neutral auditory statements were observed after citalopram treatment in paralimbic regions, the insula, striatum and prefrontal regions (Hoehn-Saric et al., 2004). However, no amygdala activation was seen in response to the studied challenges. Benzodiazepine treatment in GAD patients resulted in decreases in absolute metabolic rates for the limbic system, the basal ganglia and the cortical surface in response to an active vigilance task (Wu et al., 1991). In PTSD, fluoxetine treatment enhanced the decreased neuronal responses to war-related sounds in the prefrontal, orbitofrontal, inferior frontal and insular cortices, while attenuating enhanced responses in areas including the parietal cortex (Fernandez et al., 2001). In obsessive–compulsive disorder, normalization of challenge-induced hyperactivation of the orbitofrontal cortex has been commonly observed in different studies following behavioral or pharmacotherapy (reviewed in Cannistraro and Rauch, 2003). Taken together, these first studies have shown that aberrant activity patterns to challenges are normalized in some but not all affected brain areas following therapeutic treatment. Much more research of this type is needed before definite conclusions can be drawn, regarding which of the aberrant activity responses need to be modulated by drugs or behavioral therapy to elicit anxiolytic effects, which of those are common across different anxiety disorders and which of these responses remain treatment resistant and why.

It is not clear as yet whether psychopathological animal models can be developed for each anxiety disorder (see, e.g. Bourin, 1997), for example by choosing different selection criteria in psychogenetically selected lines. On the other hand, there is some controversy whether the current diagnostic systems, the DSM-IV (American Psychiatric Association, 1994) and the ICD-10 (World Health Organization, 1992), indeed are suited to categorizing anxiety disorders according to their underlying neurobiology. Considering also the extensive symptom overlap in these disorders, it seems to be an appropriate approach at the moment to reveal neuronal populations and mechanisms that can more generally underlie enhanced anxiety-related behavior and, in particular, to clarify how these dysfunctions can be therapeutically modulated or even reversed. Clear similarities in rodent and human findings so far are a promising basis for extending such translational research approaches in the future.

6. Conclusions and outlook

The functional mapping studies reviewed here show that the divergent anxiety-related behavioral responses of high-anxiety- vs. normal and/or low-anxiety rodents to emotional challenges are associated with differential neuronal activation in restricted parts of proposed fear/anxiety circuitries including brain areas such as the amygdala, mPFC, hippocampus, hypothalamus, lateral septum and

LC thought to be also important in stress, emotion and memory. Some of the key findings show interesting parallels to observations in human symptom provocation/challenge studies comparing anxiety disorder patients with healthy volunteers. While part of the identified differential activation in rodents followed anatomical boundaries and was found in large sections of a given brain region (e.g. the PVN), many others were found in restricted clusters in (sub)divisions of brain regions, suggesting that for this type of research, methods with high spatial resolution are preferable to other methods (e.g. Koya et al., 2005). The identification of neuronal populations showing differential activation also depends in part on the applied emotional challenge. Since different challenges appear to engage (preferentially) particular parts of the fear/anxiety circuitry, the use of an array of different challenges is essential for this research. Combining functional-mapping and tract-tracing techniques will help to establish comprehensive functional maps of the interrelationship of neuronal populations showing diverse activation. At the moment interpretations relating, for example, the observed hypoactivation in the mPFC (and hippocampus) of high-anxiety rodents to the hyperactivation in the amygdala and hypothalamus remain speculative, although there is evidence that these areas are interconnected to a considerable extent, in part via inhibitory pathways (Gray and McNaugton, 2000). Finally, it will be also very important to investigate altered neuronal activation patterns in further psychopathologically relevant rodent models of enhanced anxiety (see e.g. Table 1) and compare results with data from further human symptom provocation imaging studies involving patients with different anxiety disorders. It should then also be possible to test the hypothesis regarding whether some of these different high-anxiety animal models have particular advantages in mimicking specific human anxiety disorders.

Work to investigate exactly which of the changed neuronal activation patterns in high-anxiety rodents has to be modulated by therapeutic drugs to achieve effective anxiolysis and via which neurochemical pathways this can be accomplished, is at its early stages. Further and more extensive studies correlating (drug-induced) modulation of neuronal activation patterns with beneficial behavioral responses are needed, as well as detailed neurochemical phenotyping of the differentially activated neurons revealing the engaged neurotransmitters and receptor systems. This combined knowledge may then for example suggest that it should be of therapeutic relevance to inhibit or prevent the release of a specific neurotransmitter or the expression of specific gene products in one or more brain areas. This approach was successfully demonstrated in HAB rats concerning AVP in the PVN, a brain area showing clearly altered Fos response in these animals (Section 4.2.). It was shown by Landgraf and coworkers that a hypothalamic vasopressinergic hyperdrive contributes to the behavioral phenomenon of increased anxiety in HABs, which can be reversed by attenuating vasopressin transmission via intracerebral or intra PVN administration of an AVP V1 antagonist (Keck et al., 2003; Wigger et al., 2003). Even more selective modulation may be achieved by using tissue/region-specific knockout technology, oligonucleotide antisense for specific mRNAs or targeted drugs in order to ultimately develop new therapeutic strategies with high efficacy paired with a low side-effect profile.

Acknowledgments

The author's work cited here was supported by FWF, ÖNB, and Grants of University of Innsbruck (LFU). I would like to thank Patric Muigg for help with literature search and Rainer Landgraf (Max Planck Institute for Psychiatry, Munich) and his group for the longstanding pleasant collaboration throughout the past years.

References

Abel, E.L., Altman, H.J., Commissaris, R.L., 1992. Maudsley reactive and nonreactive rats in the forced swim test: comparison in fresh water and soiled water. Physiology & Behavior 52 (6), 1117–1119.

Abraham, I.M., Kovacs, K.J., 2000. Postnatal handling alters the activation of stress-related neuronal circuitries. European Journal of Neuroscience 12 (8), 3003–3014.

Adamec, R., Walling, S., Burton, P., 2004. Long-lasting, selective, anxiogenic effects of feline predator stress in mice. Physiology & Behavior 83 (3), 401–410.

Adamec, R.E., 2000. Evidence that long-lasting potentiation in limbic circuits mediating defensive behaviour in the right hemisphere underlies pharmacological stressor (FG-7142) induced lasting increases in anxiety-like behaviour: role of benzodiazepine receptors. Journal of Psychopharmacology 14 (4), 307–322.

Allman, J.M., Hakeem, A., Erwin, J.M., Nimchinsky, E., Hof, P., 2001. The anterior cingulate cortex. The evolution of an interface between emotion and cognition. Annals of the New York Academy of Sciences 935, 107–117.

American Psychiatric Association, 1994. Diagnostic and Statistical Manual of Mental Disorders. American Psychiatric Press, Washington, DC.

Amico, J.A., Mantella, R.C., Vollmer, R.R., Li, X., 2004. Anxiety and stress responses in female oxytocin deficient mice. Journal of Neuroendocrinology 16 (4), 319–324.

Anand, A., Shekhar, A., 2003. Brain imaging studies in mood and anxiety disorders: special emphasis on the amygdala. Annals of the New York Academy of Sciences 985, 370–388.

Andreatini, R., Blanchard, C., Blanchard, R., Brandao, M.L., Carobrez, A.P., Griebel, G., Guimaraes, F.S., Handley, S.L., Jenck, F., Leite, J.R., Rodgers, J., Schenberg, L.C., Da Cunha, C., Graeff, F.G., 2001. The brain decade in debate: II. Panic or anxiety? From animal models to a neurobiological basis. Brazilian Journal of Medical and Biological Research 34 (2), 145–154.

Arthurs, O.J., Boniface, S., 2002. How well do we understand the neural origins of the fMRI BOLD signal? Trends in Neurosciences 25 (1), 27–31.

Avgustinovich, D.F., Lipina, T.V., Bondar, N.P., Alekseyenko, O.V., Kudryavtseva, N.N., 2000. Features of the genetically defined anxiety in mice. Behavior Genetics 30 (2), 101–109.

Bakshi, V.P., Shelton, S.E., Kalin, N.H., 2000. Neurobiological correlates of defensive behaviors. Progress in Brain Research 122, 105–115.

Bannerman, D.M., Rawlins, J.N., McHugh, S.B., Deacon, R.M., Yee, B.K., Bast, T., Zhang, W.N., Pothuizen, H.H., Feldon, J., 2004.

Regional dissociations within the hippocampus—memory and anxiety. Neuroscience and Biobehavioral Reviews 28 (3), 273–283.

Barrett, D., Shumake, J., Jones, D., Gonzalez-Lima, F., 2003. Metabolic mapping of mouse brain activity after extinction of a conditioned emotional response. Journal of Neuroscience 23 (13), 5740–5749.

Battaglia, M., Ogliari, A., 2005. Anxiety and panic: from human studies to animal research and back. Neuroscience and Biobehavioral Reviews 29 (1), 169–179.

Beck, C.H., Fibiger, H.C., 1995a. Conditioned fear-induced changes in behavior and in the expression of the immediate early gene c-fos: with and without diazepam pretreatment. Journal of Neuroscience 15 (1 Pt 2), 709–720.

Beck, C.H., Fibiger, H.C., 1995b. Chronic desipramine alters stress-induced behaviors and regional expression of the immediate early gene, c-fos. Pharmacology, Biochemistry and Behavior 51 (2–3), 331–338.

Belzung, C., Griebel, G., 2001. Measuring normal and pathological anxiety-like behaviour in mice: a review. Behavioral and Brain Research 125 (1–2), 141–149.

Bernard, J.F., Bandler, R., 1998. Parallel circuits for emotional coping behaviour: new pieces in the puzzle. Journal of Comparative Neurology 401 (4), 429–436.

Blanchard, D.C., Blanchard, R.J., Lee, E.M., Nakamura, S., 1979. Defensive behaviors in rats following septal and septal-amygdala lesions. Journal of Comparative and Physiological Psychology 93 (2), 378–390.

Blanchard, D.C., Griebel, G., Blanchard, R.J., 2001. Mouse defensive behaviors: pharmacological and behavioral assays for anxiety and panic. Neuroscience and Biobehavioral Reviews 25 (3), 205–218.

Blanchard, D.C., Canteras, N.S., Markham, C.M., Pentkowski, N.S., Blanchard, R.J., 2005. Lesions of structures showing FOS expression to cat presentation: effects on responsivity to a Cat, Cat odor, and nonpredator threat. Neuroscience and Biobehavioral Reviews 29 (8), 1243–1253.

Blizard, D.A., Adams, N., 2002. The maudsley reactive and nonreactive strains: a new perspective. Behavior Genetics 32 (5), 277–299.

Bosch, O.J., Meddle, S.L., Beiderbeck, D.I., Douglas, A.J., Neumann, I.D., 2005. Brain oxytocin correlates with maternal aggression: link to anxiety. Journal of Neuroscience 25 (29), 6807–6815.

Boshuisen, M.L., Ter Horst, G.J., Paans, A.M., Reinders, A.A., den Boer, J.A., 2002. rCBF differences between panic disorder patients and control subjects during anticipatory anxiety and rest. Biological Psychiatry 52 (2), 126–135.

Bourin, M., 1997. Animal models of anxiety: are they suitable for predicting drug action in humans? Polish Journal of Pharmacology 49 (2–3), 79–84.

Bouwknecht, J.A., Paylor, R., 2002. Behavioral and physiological mouse assays for anxiety: a survey in nine mouse strains. Behavioral and Brain Research 136 (2), 489–501.

Brandao, M.L., Borelli, K.G., Nobre, M.J., Santos, J.M., Albrechet-Souza, L., Oliveira, A.R., Martinez, R.C., 2005. Gabaergic regulation of the neural organization of fear in the midbrain tectum. Neuroscience and Biobehavioral Reviews.

Bremner, J.D., 2004. Brain imaging in anxiety disorders. Expert Review of Neurotherapy 4 (2), 275–284.

Broadhurst, P.L., 1960. Applications of biometrical genetics to the inheritance of behaviour. In: Eysenk, H.J. (Ed.), Experiments in Personality. Routledge Kegan Paul, London, pp. 1–102.

Brunelli, S.A., 2005. Selective breeding for an infant phenotype: rat pup ultrasonic vocalization (USV). Behavior Genetics 35 (1), 53–65.

Brush, F.R., 2003. Selection for differences in avoidance learning: the Syracuse strains differ in anxiety, not learning ability. Behavior Genetics 33 (6), 677–696.

Caldji, C., Francis, D., Sharma, S., Plotsky, P.M., Meaney, M.J., 2000. The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. Neuropsychopharmacology 22 (3), 219–229.

Campeau, S., Falls, W.A., Cullinan, W.E., Helmreich, D.L., Davis, M., Watson, S.J., 1997. Elicitation and reduction of fear: behavioural and neuroendocrine indices and brain induction of the immediate-early gene c-fos. Neuroscience 78 (4), 1087–1104.

Cannistraro, P.A., Rauch, S.L., 2003. Neural circuitry of anxiety: evidence from structural and functional neuroimaging studies. Psychopharmacology Bulletin 37 (4), 8–25.

Canteras, N.S., 2002. The medial hypothalamic defensive system: hodological organization and functional implications. Pharmacology, Biochemistry and Behavior 71 (3), 481–491.

Canteras, N.S., Chiavegatto, S., Valle, L.E., Swanson, L.W., 1997. Severe reduction of rat defensive behavior to a predator by discrete hypothalamic chemical lesions. Brain Research Bulletin 44 (3), 297–305.

Carrie, I., Clement, M., de Javel, D., Frances, H., Bourre, J.M., 2000. Phospholipid supplementation reverses behavioral and biochemical alterations induced by n-3 polyunsaturated fatty acid deficiency in mice. Journal of Lipid Research 41 (3), 473–480.

Ceccatelli, S., Villar, M.J., Goldstein, M., Hokfelt, T., 1989. Expression of c-Fos immunoreactivity in transmitter-characterized neurons after stress. Proceedings of the National Academy of Sciences USA 86 (23), 9569–9573.

Cecchi, G.A., Ribeiro, S., Mello, C.V., Magnasco, M.O., 1999. An automated system for the mapping and quantitative analysis of immunocytochemistry of an inducible nuclear protein. Journal of Neuroscience Methods 87 (2), 147–158.

Chan, R.K., Brown, E.R., Ericsson, A., Kovacs, K.J., Sawchenko, P.E., 1993. A comparison of two immediate-early genes, c-fos and NGFI-B, as markers for functional activation in stress-related neuroendocrine circuitry. Journal of Neuroscience 13 (12), 5126–5138.

Charney, D.S., 1998. Monoamine dysfunction and the pathophysiology and treatment of depression. Journal of Clinical Psychiatry 59 (Suppl. 14), 11–14.

Charney, D.S., 2003. Neuroanatomical circuits modulating fear and anxiety behaviors. Acta Psychiatrica Scandinavica, Supplementum (417), 38–50.

Chaudhuri, A., 1997. Neural activity mapping with inducible transcription factors. Neuroreport 8 (16), v–ix.

Chaudhuri, A., Zangenehpour, S., 2002. Molecular activity maps of sensory function. In: Kaczmarek, L., Robertson, H.A. (Eds.), Immediate Early Genes and Inducible Transcription Factors in Mapping of the Central Nervous System Function and Dysfunction. Elsevier, Amsterdam, pp. 103–145.

Chaudhuri, A., Nissanov, J., Larocque, S., Rioux, L., 1997. Dual activity maps in primate visual cortex produced by different temporal patterns of zif268 mRNA and protein expression. Proceedings of the National Academy of Sciences USA 94 (6), 2671–2675.

Chung, S., Son, G.H., Park, S.H., Park, E., Lee, K.H., Geum, D., Kim, K., 2005. Differential adaptive responses to chronic stress of maternally stressed male mice offspring. Endocrinology 146 (7), 3202–3210.

Clement, Y., Calatayud, F., Belzung, C., 2002. Genetic basis of anxiety-like behaviour: a critical review. Brain Research Bulletin 57 (1), 57–71.

Conversi, D., Orsini, C., Cabib, S., 2004. Distinct patterns of Fos expression induced by systemic amphetamine in the striatal complex of C57BL/6JICo and DBA/2JICo inbred strains of mice. Brain Research 1025 (1–2), 59–66.

Correll, C.M., Rosenkranz, J.A., Grace, A.A., 2005. Chronic cold stress alters prefrontal cortical modulation of amygdala neuronal activity in rats. Biological Psychiatry 58 (5), 382–391.

Crabbe, J.C., Wahlsten, D., Dudek, B.C., 1999. Genetics of mouse behavior: interactions with laboratory environment. Science 284 (5420), 1670–1672.

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 Research 835 (1), 18–26.

Cryan, J.F., Holmes, A., 2005. The ascent of mouse: advances in modelling human depression and anxiety. Nature Reviews Drug Discovery 4 (9), 775–790.

Cullinan, W.E., Herman, J.P., Battaglia, D.F., Akil, H., Watson, S.J., 1995. Pattern and time course of immediate early gene expression in rat brain following acute stress. Neuroscience 64 (2), 477–505.

D'Aquila, P.S., Brain, P., Willner, P., 1994. Effects of chronic mild stress on performance in behavioural tests relevant to anxiety and depression. Physiology & Behavior 56 (5), 861–867.

Davidson, R.J., Lewis, D.A., Alloy, L.B., Amaral, D.G., Bush, G., Cohen, J.D., Drevets, W.C., Farah, M.J., Kagan, J., McClelland, J.L., Nolen-Hoeksema, S., Peterson, B.S., 2002. Neural and behavioral substrates of mood and mood regulation. Biological Psychiatry 52 (6), 478–502.

Dayas, C.V., Buller, K.M., Day, T.A., 1999. Neuroendocrine responses to an emotional stressor: evidence for involvement of the medial but not the central amygdala. European Journal of Neuroscience 11 (7), 2312–2322.

de Medeiros, M.A., Carlos Reis, L., Eugenio Mello, L., 2005. Stress-induced c-Fos expression is differentially modulated by dexamethasone, diazepam and imipramine. Neuropsychopharmacology 30 (7), 1246–1256.

Degroot, A., Kashluba, S., Treit, D., 2001. Septal GABAergic and hippocampal cholinergic systems modulate anxiety in the plus-maze and shock-probe tests. Pharmacology, Biochemistry and Behavior 69 (3–4), 391–399.

Dichter, G.S., Brunelli, S.A., Hofer, M.A., 1996. Elevated plus-maze behavior in adult offspring of selectively bred rats. Physiology & Behavior 60 (1), 299–304.

Dielenberg, R.A., McGregor, I.S., 2001. Defensive behavior in rats towards predatory odors: a review. Neuroscience and Biobehavioral Reviews 25 (7–8), 597–609.

Dielenberg, R.A., Hunt, G.E., McGregor, I.S., 2001. 'When a rat smells a cat': the distribution of Fos immunoreactivity in rat brain following exposure to a predatory odor. Neuroscience 104 (4), 1085–1097.

Dorow, R., Horowski, R., Paschelke, G., Amin, M., 1983. Severe anxiety induced by FG 7142, a beta-carboline ligand for benzodiazepine receptors. Lancet 2 (8341), 98–99.

Dragunow, M., Faull, R., 1989. The use of c-fos as a metabolic marker in neuronal pathway tracing. Journal of Neuroscience Methods 29 (3), 261–265.

Drevets, W.C., 2001. Neuroimaging and neuropathological studies of depression: implications for the cognitive–emotional features of mood disorders. Current Opinion in Neurobiology 11 (2), 240–249.

Driscoll, P., Escorihuela, R.M., Fernandez-Teruel, A., Giorgi, O., Schwegler, H., Steimer, T., Wiersma, A., Corda, M.G., Flint, J., Koolhaas, J.M., Langhans, W., Schulz, P.E., Siegel, J., Tobena, A., 1998. Genetic selection and differential stress responses. The Roman lines/strains of rats. Annals of the New York Academy of Sciences 851, 501–510.

Duncan, G.E., Johnson, K.B., Breese, G.R., 1993. Topographic patterns of brain activity in response to swim stress: assessment by 2-deoxyglucose uptake and expression of Fos-like immunoreactivity. Journal of Neuroscience 13 (9), 3932–3943.

Duncan, G.E., Knapp, D.J., Breese, G.R., 1996a. Neuroanatomical characterization of Fos induction in rat behavioral models of anxiety. Brain Research 713 (1–2), 79–91.

Duncan, G.E., Knapp, D.J., Johnson, K.B., Breese, G.R., 1996b. Functional classification of antidepressants based on antagonism of swim stress-induced fos-like immunoreactivity. Journal of Pharmacology and Experimental Therapeutics 277 (2), 1076–1089.

Ebner, K., Rupniak, N.M., Saria, A., Singewald, N., 2004. Substance P in the medial amygdala: emotional stress-sensitive release and modulation of anxiety-related behavior in rats. Proceedings of the National Academy of Sciences USA 101 (12), 4280–4285.

Emmert, M.H., Herman, J.P., 1999. Differential forebrain c-fos mRNA induction by ether inhalation and novelty: evidence for distinctive stress pathways. Brain Research 845 (1), 60–67.

Estanislau, C., Morato, S., 2005. Prenatal stress produces more behavioral alterations than maternal separation in the elevated plus-maze and in the elevated T-maze. Behavioral and Brain Research 163 (1), 70–77.

Feldman, S., Conforti, N., Saphier, D., 1990. The preoptic area and bed nucleus of the stria terminalis are involved in the effects of the amygdala on adrenocortical secretion. Neuroscience 37 (3), 775–779.

Fernandez, M., Pissiota, A., Frans, O., von Knorring, L., Fischer, H., Fredrikson, M., 2001. Brain function in a patient with torture related post-traumatic stress disorder before and after fluoxetine treatment: a positron emission tomography provocation study. Neuroscience Letters 297 (2), 101–104.

Fichter, M.M., Narrow, W.E., Roper, M.T., Rehm, J., Elton, M., Rae, D.S., Locke, B.Z., Regier, D.A., 1996. Prevalence of mental illness in Germany and the United States. Comparison of the Upper Bavarian Study and the Epidemiologic Catchment Area Program. Journal of Nervous and Mental Disease 184 (10), 598–606.

File, S.E., 2001. Factors controlling measures of anxiety and responses to novelty in the mouse. Behavioral and Brain Research 125 (1–2), 151–157.

Finn, D.A., Rutledge-Gorman, M.T., Crabbe, J.C., 2003. Genetic animal models of anxiety. Neurogenetics 4 (3), 109–135.

Francis, D.D., Diorio, J., Plotsky, P.M., Meaney, M.J., 2002. Environmental enrichment reverses the effects of maternal separation on stress reactivity. Journal of Neuroscience 22 (18), 7840–7843.

Frank, E., Salchner, P., Aldag, J.M., Salome, N., Singewald, N., Landgraf, R., Wigger, A., 2006. Genetic predisposition to anxiety-related behavior determines coping style, neuroendocrine responses, and neuronal activation during social defeat. Behavioral Neuroscience 120, 60–71.

Fredrikson, M., Furmark, T., 2003. Amygdaloid regional cerebral blood flow and subjective fear during symptom provocation in anxiety disorders. Annals of the New York Academy of Sciences 985, 341–347.

Fredrikson, M., Wik, G., Fischer, H., Andersson, J., 1995. Affective and attentive neural networks in humans: a PET study of Pavlovian conditioning. Neuroreport 7 (1), 97–101.

Fujita, O., Annen, Y., Kitaoka, A., 1994. Tsukuba high- and low-emotional strains of rats (*Rattus norvegicus*): an overview. Behavior Genetics 24 (4), 389–415.

Furmark, T., Tillfors, M., Marteinsdottir, I., Fischer, H., Pissiota, A., Langstrom, B., Fredrikson, M., 2002. Common changes in cerebral blood flow in patients with social phobia treated with citalopram or cognitive–behavioral therapy. Archives of General Psychiatry 59 (5), 425–433.

Furmark, T., Appel, L., Michelgard, A., Wahlstedt, K., Ahs, F., Zancan, S., Jacobsson, E., Flyckt, K., Grohp, M., Bergstrom, M., Pich, E.M., Nilsson, L.G., Bani, M., Langstrom, B., Fredrikson, M., 2005. Cerebral blood flow changes after treatment of social phobia with the neurokinin-1 antagonist GR205171, citalopram, or placebo. Biological Psychiatry 58 (2), 132–142.

Gass, P., Fleischmann, A., Hvalby, O., Jensen, V., Zacher, C., Strekalova, T., Kvello, A., Wagner, E.F., Sprengel, R., 2004. Mice with a fra-1 knock-in into the c-fos locus show impaired spatial but regular contextual learning and normal LTP. Molecular Brain Research 130 (1–2), 16–22.

Ghosh, A., Ginty, D.D., Bading, H., Greenberg, M.E., 1994. Calcium regulation of gene expression in neuronal cells. Journal of Neurobiology 25 (3), 294–303.

Girotti, M., Pace, T.W., Gaylord, R.I., Rubin, B.A., Herman, J.P., Spencer, R.L., 2006. Habituation to repeated restraint stress is associated with lack of stress-induced c-fos expression in primary sensory processing areas of the rat brain. Neuroscience.

Goddard, A.W., Charney, D.S., 1997. Toward an integrated neurobiology of panic disorder. Journal of Clinical Psychiatry 58 (Suppl. 2), 4–11.

Gorman, J.M., Kent, J.M., Sullivan, G.M., Coplan, J.D., 2000. Neuroanatomical hypothesis of panic disorder, revised. American Journal of Psychiatry 157 (4), 493–505.

Gorwood, P., 2004. Generalized anxiety disorder and major depressive disorder comorbidity: an example of genetic pleiotropy? European Psychiatry 19 (1), 27–33.

Gotoh, J., Kuang, T.Y., Nakao, Y., Cohen, D.M., Melzer, P., Itoh, Y., Pak, H., Pettigrew, K., Sokoloff, L., 2001. Regional differences in mechanisms of cerebral circulatory response to neuronal activation. American Journal of Physiology—Heart and Circulatory Physiology 280 (2), H821–H829.

Gray, J., McNaugton, N., 2000. The Neuropsychology of Anxiety. Oxford University Press, Oxford.

Greenberg, M.E., Ziff, E.B., 1984. Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene. Nature 311 (5985), 433–438.

Griebel, G., Blanchard, D.C., Jung, A., Lee, J.C., Masuda, C.K., Blanchard, R.J., 1995. Further evidence that the mouse defense test battery is useful for screening anxiolytic and panicolytic drugs: effects of acute and chronic treatment with alprazolam. Neuropharmacology 34 (12), 1625–1633.

Gutman, D.A., Nemeroff, C.B., 2003. Persistent central nervous system effects of an adverse early environment: clinical and preclinical studies. Physiology & Behavior 79 (3), 471–478.

Guzowski, J.F., 2002. Insights into immediate-early gene function in hippocampal memory consolidation using antisense oligonucleotide and fluorescent imaging approaches. Hippocampus 12 (1), 86–104.

Guzowski, J.F., McNaughton, B.L., Barnes, C.A., Worley, P.F., 2001. Imaging neural activity with temporal and cellular resolution using FISH. Current Opinion in Neurobiology 11 (5), 579–584.

Guzowski, J.F., Timlin, J.A., Roysam, B., McNaughton, B.L., Worley, P.F., Barnes, C.A., 2005. Mapping behaviorally relevant neural circuits with immediate-early gene expression. Current Opinion in Neurobiology 15 (5), 599–606.

Harro, J., Haidkind, R., Harro, M., Modiri, A.R., Gillberg, P.G., Pahkla, R., Matto, V., Oreland, L., 1999. Chronic mild unpredictable stress after noradrenergic denervation: attenuation of behavioural and biochemical effects of DSP-4 treatment. European Neuropsychopharmacology 10 (1), 5–16.

Heidbreder, C.A., Groenewegen, H.J., 2003. The medial prefrontal cortex in the rat: evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. Neuroscience and Biobehavioral Reviews 27 (6), 555–579.

Henniger, M.S., Ohl, F., Holter, S.M., Weissenbacher, P., Toschi, N., Lorscher, P., Wigger, A., Spanagel, R., Landgraf, R., 2000. Unconditioned anxiety and social behaviour in two rat lines selectively bred for high and low anxiety-related behaviour. Behavioral and Brain Research 111 (1–2), 153–163.

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 Research Reviews 28 (3), 370–490.

Herdegen, T., Kovary, K., Buhl, A., Bravo, R., Zimmermann, M., Gass, P., 1995. Basal expression of the inducible transcription factors c-Jun, JunB, JunD, c-Fos, FosB, and Krox-24 in the adult rat brain. Journal of Comparative Neurology 354 (1), 39–56.

Herman, J.P., Ostrander, M.M., Mueller, N.K., Figueiredo, H., 2005. Limbic system mechanisms of stress regulation: hypothalamo–pituitary–adrenocortical axis. Progress in Neuropsychopharmacology & Biological Psychiatry 29 (8), 1201–1213.

Herrera, D.G., Robertson, H.A., 1996. Activation of c-fos in the brain. Progress in Neurobiology 50 (2–3), 83–107.

Hetzenauer, A., Muigg, P., Landgraf, R., Singewald, N., 2005. Differential extinction of cued conditioned fear memory in rats selectively bred for high, normal and low anxiety. Journal of Neurochemistry 94 (2), 401.

Hitzemann, B., Hitzemann, R., 1999. Chlordiazepoxide-induced expression of c-Fos in the central extended amygdala and other brain regions of the C57BL/6J and DBA/2J inbred mouse strains: relationships to mechanisms of ethanol action. Alcohol—Clinical and Experimenatal Research 23 (7), 1158–1172.

Hoehn-Saric, R., Schlund, M.W., Wong, S.H., 2004. Effects of citalopram on worry and brain activation in patients with generalized anxiety disorder. Psychiatry Research 131 (1), 11–21.

Hoffman, G.E., Lyo, D., 2002. Anatomical markers of activity in neuroendocrine systems: are we all 'fos-ed out'? Journal of Neuroendocrinology 14 (4), 259–268.

Holmes, A., le Guisquet, A.M., Vogel, E., Millstein, R.A., Leman, S., Belzung, C., 2005. Early life genetic, epigenetic and environmental factors shaping emotionality in rodents. Neuroscience and Biobehavioral Reviews 29 (8), 1335–1346.

Holschneider, D.P., Yang, J., Sadler, T.R., Nguyen, P.T., Givrad, T.K., Maarek, J.M., 2005. Mapping cerebral blood flow changes during auditory-cued conditioned fear in the nontethered, nonrestrained rat. Neuroimage.

Horowski, R., Dorrow, R., 2002. Anxiogenic, not psychotogenic, properties of the partial inverse benzodiazepine receptor agonist FG 7142 in man. Psychopharmacology (Berl) 162 (2), 223–224.

Ikeda, J., Nakajima, T., Osborne, O.C., Mies, G., Nowak Jr., T.S., 1994. Coexpression of c-fos and hsp70 mRNAs in gerbil brain after ischemia: induction threshold, distribution and time course evaluated by in situ hybridization. Brain Research and Molecular Brain Research 26 (1–2), 249–258.

Ikeda, K., Onaka, T., Yamakado, M., Nakai, J., Ishikawa, T.O., Taketo, M.M., Kawakami, K., 2003. Degeneration of the amygdala/piriform cortex and enhanced fear/anxiety behaviors in sodium pump alpha2 subunit (Atp1a2)-deficient mice. Journal of Neuroscience 23 (11), 4667–4676.

Jenck, F., Moreau, J.L., Martin, J.R., 1995. Dorsal periaqueductal gray-induced aversion as a simulation of panic anxiety: elements of face and predictive validity. Psychiatry Research 57 (2), 181–191.

Jinks, A.L., McGregor, I.S., 1997. Modulation of anxiety-related behaviours following lesions of the prelimbic or infralimbic cortex in the rat. Brain Research 772 (1–2), 181–190.

Kabbaj, M., Akil, H., 2001. Individual differences in novelty-seeking behavior in rats: a c-fos study. Neuroscience 106 (3), 535–545.

Kalin, N.H., Shelton, S.E., Davidson, R.J., 2004. The role of the central nucleus of the amygdala in mediating fear and anxiety in the primate. Journal of Neuroscience 24 (24), 5506–5515.

Kalisch, R., Salome, N., Platzer, S., Wigger, A., Czisch, M., Sommer, W., Singewald, N., Heilig, M., Berthele, A., Holsboer, F., Landgraf, R., Auer, D.P., 2004. High trait anxiety and hyporeactivity to stress of the dorsomedial prefrontal cortex: a combined phMRI and Fos study in rats. Neuroimage 23 (1), 382–391.

Kalueff, A.V., Tuohimaa, P., 2004. Experimental modeling of anxiety and depression. Acta Neurobiologiae Experimentalis (Wars) 64 (4), 439–448.

Kalueff, A.V., Tuohimaa, P., 2005. Contrasting grooming phenotypes in three mouse strains markedly different in anxiety and activity (129S1, BALB/c and NMRI). Behavioral and Brain Research 160 (1), 1–10.

Kantor, S., Anheuer, Z.E., Bagdy, G., 2000. High social anxiety and low aggression in Fawn-Hooded rats. Physiology & Behavior 71 (5), 551–557.

Kask, A., Harro, J., von Horsten, S., Redrobe, J.P., Dumont, Y., Quirion, R., 2002. The neurocircuitry and receptor subtypes mediating anxiolytic-like effects of neuropeptide Y. Neuroscience and Biobehavioral Reviews 26 (3), 259–283.

Keay, K.A., Bandler, R., 2001. Parallel circuits mediating distinct emotional coping reactions to different types of stress. Neuroscience and Biobehavioral Reviews 25 (7–8), 669–678.

Keck, M.E., Wigger, A., Welt, T., Muller, M.B., Gesing, A., Reul, J.M., Holsboer, F., Landgraf, R., Neumann, I.D., 2002. Vasopressin mediates the response of the combined dexamethasone/CRH test in hyper-anxious rats: implications for pathogenesis of affective disorders. Neuropsychopharmacology 26 (1), 94–105.

Keck, M.E., Welt, T., Muller, M.B., Uhr, M., Ohl, F., Wigger, A., Toschi, N., Holsboer, F., Landgraf, R., 2003. Reduction of hypothalamic vasopressinergic hyperdrive contributes to clinically relevant behavioral and neuroendocrine effects of chronic paroxetine

treatment in a psychopathological rat model. Neuropsychopharmacology 28 (2), 235–243.
Keck, M.E., Sartori, S.B., Welt, T., Muller, M.B., Ohl, F., Holsboer, F., Landgraf, R., Singewald, N., 2005. Differences in serotonergic neurotransmission between rats displaying high or low anxiety/depression-like behaviour: effects of chronic paroxetine treatment. Journal of Neurochemistry 92 (5), 1170–1179.
Kendler, K.S., Neale, M.C., Kessler, R.C., Heath, A.C., Eaves, L.J., 1992. Major depression and generalized anxiety disorder. Same genes, (partly) different environments? Archives of General Psychiatry 49 (9), 716–722.
Kent, J.M., Rauch, S.L., 2003. Neurocircuitry of anxiety disorders. Current Psychiatry Reports 5 (4), 266–273.
Kessler, R.C., McGonagle, K.A., Zhao, S., Nelson, C.B., Hughes, M., Eshleman, S., Wittchen, H.U., Kendler, K.S., 1994. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. Archives of General Psychiatry 51 (1), 8–19.
Kessler, R.M., 2003. Imaging methods for evaluating brain function in man. Neurobiology of Aging 24 (Suppl. 1), S21–S35 (discussion S37-9).
King, S.M., 1999. Escape-related behaviours in an unstable elevated and exposed environment. I. A new behavioural model of extreme anxiety. Behavioral and Brain Research 98 (1), 113–126.
Kishimoto, T., Radulovic, J., Radulovic, M., Lin, C.R., Schrick, C., Hooshmand, F., Hermanson, O., Rosenfeld, M.G., Spiess, J., 2000. Deletion of crhr2 reveals an anxiolytic role for corticotropin-releasing hormone receptor-2. Nature Genetics 24 (4), 415–419.
Kitaoka, A., Fujita, O., 1991. Behavioral comparisons of the Tsukuba emotional strains of rats (*Rattus norvegicus*) in three types of novel situations. Behavior Genetics 21 (3), 317–325.
Knapp, D.J., Duncan, G.E., Crews, F.T., Breese, G.R., 1998. Induction of Fos-like proteins and ultrasonic vocalizations during ethanol withdrawal: further evidence for withdrawal-induced anxiety. Alcohol—Clinical and Experimenatal Research 22 (2), 481–493.
Kovacs, K.J., 1998. c-Fos as a transcription factor: a stressful (re)view from a functional map. Neurochemistry International 33 (4), 287–297.
Kovacs, K.J., Sawchenko, P.E., 1996. Sequence of stress-induced alterations in indices of synaptic and transcriptional activation in parvocellular neurosecretory neurons. Journal of Neuroscience 16 (1), 262–273.
Koya, E., Spijker, S., Homberg, J.R., Voorn, P., Schoffelmeer, A.N., De Vries, T.J., Smit, A.B., 2005. Molecular reactivity of mesocorticolimbic brain areas of high and low grooming rats after elevated plus maze exposure. Brain Research and Molecular Brain Research 137 (1–2), 184–192.
Kromer, S.A., Kessler, M.S., Milfay, D., Birg, I.N., Bunck, M., Czibere, L., Panhuysen, M., Putz, B., Deussing, J.M., Holsboer, F., Landgraf, R., Turck, C.W., 2005. Identification of glyoxalase-I as a protein marker in a mouse model of extremes in trait anxiety. Journal of Neuroscience 25 (17), 4375–4384.
Kubota, O., Hattori, K., Hashimoto, K., Yagi, T., Sato, T., Iyo, M., Yuasa, S., 2004. Auditory-conditioned-fear-dependent c-Fos expression is altered in the emotion-related brain structures of Fyn-deficient mice. Brain Research and Molecular Brain Research 130 (1–2), 149–160.
Lahti, K.M., Ferris, C.F., Li, F., Sotak, C.H., King, J.A., 1998. Imaging brain activity in conscious animals using functional MRI. Journal of Neuroscience Methods 82 (1), 75–83.
Lamprea, M.R., Cardenas, F.P., Vianna, D.M., Castilho, V.M., Cruz-Morales, S.E., Brandao, M.L., 2002. The distribution of fos immunoreactivity in rat brain following freezing and escape responses elicited by electrical stimulation of the inferior colliculus. Brain Research 950 (1–2), 186–194.
Landgraf, R., Wigger, A., 2002. High vs low anxiety-related behavior rats: an animal model of extremes in trait anxiety. Behavior Genetics 32 (5), 301–314.
Landgraf, R., Wigger, A., 2003. Born to be anxious: neuroendocrine and genetic correlates of trait anxiety in HAB rats. Stress 6 (2), 111–119.
Landgraf, R., Keßler, M., Bunck, M., Murgatroyd, C., Spengler, D., Zimbelmann, M., Nußbaumer, M., Singewald, N., Rujescu, D., Frank, E., 2006. Candidate genes of anxiety and depression-like behavior and their products in HAB/LAB rats and mice. Neuroscience and Biobehavioral Reviews, in press.
Lawrence, J., Stroman, P.W., Bascaramurty, S., Jordan, L.M., Malisza, K.L., 2004. Correlation of functional activation in the rat spinal cord with neuronal activation detected by immunohistochemistry. Neuroimage 22 (4), 1802–1807.
Lazovic, J., Wrzos, H.F., Yang, Q.X., Collins, C.M., Smith, M.B., Norgren, R., Matyas, K., Ouyang, A., 2005. Regional activation in the rat brain during visceral stimulation detected by c-fos expression and fMRI. Neurogastroenterology and Motility 17 (4), 548–556.
Lesch, K.P., Zeng, Y., Reif, A., Gutknecht, L., 2003. Anxiety-related traits in mice with modified genes of the serotonergic pathway. European Journal of Pharmacology 480 (1–3), 185–204.
Levine, S., 2002. Regulation of the hypothalamic–pituitary–adrenal axis in the neonatal rat: the role of maternal behavior. Neurotoxicity Research 4 (5–6), 557–564.
Li, C.I., Maglinao, T.L., Takahashi, L.K., 2004. Medial amygdala modulation of predator odor-induced unconditioned fear in the rat. Behavioral Neuroscience 118 (2), 324–332.
Liebsch, G., Montkowski, A., Holsboer, F., Landgraf, R., 1998. Behavioural profiles of two Wistar rat lines selectively bred for high or low anxiety-related behaviour. Behavioral and Brain Research 94 (2), 301–310.
Linden, A.M., Baez, M., Bergeron, M., Schoepp, D.D., 2003. Increased c-Fos expression in the centromedial nucleus of the thalamus in metabotropic glutamate 8 receptor knockout mice following the elevated plus maze test. Neuroscience 121 (1), 167–178.
Lino-de-Oliveira, C., Sales, A.J., Del Bel, E.A., Silveira, M.C., Guimaraes, F.S., 2001. Effects of acute and chronic fluoxetine treatments on restraint stress-induced Fos expression. Brain Research Bulletin 55 (6), 747–754.
Lorberbaum, J.P., Kose, S., Johnson, M.R., Arana, G.W., Sullivan, L.K., Hamner, M.B., Ballenger, J.C., Lydiard, R.B., Brodrick, P.S., Bohning, D.E., George, M.S., 2004. Neural correlates of speech anticipatory anxiety in generalized social phobia. Neuroreport 15 (18), 2701–2705.
Lovick, T.A., 1996. Midbrain and medullary regulation of defensive cardiovascular functions. Progress in Brain Research 107, 301–313.
Lu, H., Patel, S., Luo, F., Li, S.J., Hillard, C.J., Ward, B.D., Hyde, J.S., 2004. Spatial correlations of laminar BOLD and CBV responses to rat whisker stimulation with neuronal activity localized by Fos expression. Magnetic Resonance in Medicine 52 (5), 1060–1068.
Lu, X.H., Li, L.J., Li, C.Q., Luo, X.G., 2000. Potential anxiolytic role of c-fos antisense oligonucleotide in social-defeated rats. Hunan Yi Ke Da Xue Xue Bao 25 (6), 535–538.
Lukasik, V.M., Gillies, R.J., 2003. Animal anaesthesia for in vivo magnetic resonance. NMR Biomedicine 16 (8), 459–467.
Lyford, G.L., Yamagata, K., Kaufmann, W.E., Barnes, C.A., Sanders, L.K., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Lanahan, A.A., Worley, P.F., 1995. Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. Neuron 14 (2), 433–445.
Ma, S., Morilak, D.A., 2004. Induction of FOS expression by acute immobilization stress is reduced in locus coeruleus and medial amygdala of Wistar–Kyoto rats compared to Sprague–Dawley rats. Neuroscience 124 (4), 963–972.
Ma, S., Morilak, D.A., 2005. Norepinephrine release in medial amygdala facilitates activation of the hypothalamic–pituitary–adrenal axis in response to acute immobilisation stress. Journal of Neuroendocrinology 17 (1), 22–28.
Malizia, A.L., 1999. What do brain imaging studies tell us about anxiety disorders? Journal of Psychopharmacology 13 (4), 372–378.

Malkani, S., Rosen, J.B., 2000. Differential expression of EGR-1 mRNA in the amygdala following diazepam in contextual fear conditioning. Brain Research 860 (1–2), 53–63.

Mantella, R.C., Vollmer, R.R., Rinaman, L., Li, X., Amico, J.A., 2004. Enhanced corticosterone concentrations and attenuated Fos expression in the medial amygdala of female oxytocin knockout mice exposed to psychogenic stress. American Journal of Physiology—Regulatory Integrative and Comparative Physiology 287 (6), R1494–R1504.

Maren, S., 2005. Building and burying fear memories in the brain. Neuroscientist 11 (1), 89–99.

Maren, S., Quirk, G.J., 2004. Neuronal signalling of fear memory. Nature Reviews Neuroscience 5 (11), 844–852.

Marshall, R.D., Spitzer, R., Liebowitz, M.R., 1999. Review and critique of the new DSM-IV diagnosis of acute stress disorder. American Journal o f Psychiatry 156 (11), 1677–1685.

Martinez, M., Calvo-Torrent, A., Herbert, J., 2002. Mapping brain response to social stress in rodents with c-fos expression: a review. Stress 5 (1), 3–13.

Maslova, L.N., Bulygina, V.V., Markel, A.L., 2002. Chronic stress during prepubertal development: immediate and long-lasting effects on arterial blood pressure and anxiety-related behavior. Psychoneuroendocrinology 27 (5), 549–561.

McClung, C.A., Ulery, P.G., Perrotti, L.I., Zachariou, V., Berton, O., Nestler, E.J., 2004. DeltaFosB: a molecular switch for long-term adaptation in the brain. Brain Research and Molecular Brain Research 132 (2), 146–154.

McGregor, I.S., Hargreaves, G.A., Apfelbach, R., Hunt, G.E., 2004. Neural correlates of cat odor-induced anxiety in rats: region-specific effects of the benzodiazepine midazolam. Journal of Neuroscience 24 (17), 4134–4144.

McIntosh, J., Anisman, H., Merali, Z., 1999. Short- and long-periods of neonatal maternal separation differentially affect anxiety and feeding in adult rats: gender-dependent effects. Brain Research and Developemental Brain Research 113 (1–2), 97–106.

Menard, J., Treit, D., 1999. Effects of centrally administered anxiolytic compounds in animal models of anxiety. Neuroscience and Biobehavioral Reviews 23 (4), 591–613.

Menon, R.S., Gati, J.S., Goodyear, B.G., Luknowsky, D.C., Thomas, C.G., 1998. Spatial and temporal resolution of functional magnetic resonance imaging. Biochemistry and Cell Biology 76 (2–3), 560–571.

Millan, M.J., 2003. The neurobiology and control of anxious states. Progress in Neurobiology 70 (2), 83–244.

Million, M., Wang, L., Martinez, V., Tache, Y., 2000. Differential Fos expression in the paraventricular nucleus of the hypothalamus, sacral parasympathetic nucleus and colonic motor response to water avoidance stress in Fischer and Lewis rats. Brain Research 877 (2), 345–353.

Mintun, M.A., Lundstrom, B.N., Snyder, A.Z., Vlassenko, A.G., Shulman, G.L., Raichle, M.E., 2001. Blood flow and oxygen delivery to human brain during functional activity: theoretical modeling and experimental data. Proceedings of the National Academy of Sciences USA 98 (12), 6859–6864.

Morgan, J.I., Curran, T., 1991. Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. Annual Review of Neuroscience 14, 421–451.

Morgan, J.I., Cohen, D.R., Hempstead, J.L., Curran, T., 1987. Mapping patterns of c-fos expression in the central nervous system after seizure. Science 237 (4811), 192–197.

Morgan, M.A., LeDoux, J.E., 1995. Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. Behavioral Neuroscience 109 (4), 681–688.

Morinobu, S., Nibuya, M., Duman, R.S., 1995. Chronic antidepressant treatment down-regulates the induction of c-fos mRNA in response to acute stress in rat frontal cortex. Neuropsychopharmacology 12 (3), 221–228.

Muigg, P., Wigger, A., Singewald, N., 2004. Chronic paroxetine treatment modulates the neuronal activation pattern in HAB rats in response to the forced swim test. Naunyn-Schmiedeberg's Archives of Pharmacology 369 (Suppl. 1), R87.

Muigg, P., Salchner, P., Scheiber, S., Bunck, M., Landgraf, R., Singewald, N., 2005. Differential stress-induced neuronal activation pattern in mouse lines selectively bred for high, normal and low anxiety. Journal of Neurochemistry 94 (Suppl. 2), 421.

Mulders, N., Chan, M.H., 1995. Heat capacity measurements of 3He–4He mixtures in aerogel. Physical Review Letters 75 (20), 3705–3708.

Muller, M.B., Zimmermann, S., Sillaber, I., Hagemeyer, T.P., Deussing, J.M., Timpl, P., Kormann, M.S., Droste, S.K., Kuhn, R., Reul, J.M., Holsboer, F., Wurst, W., 2003. Limbic corticotropin-releasing hormone receptor 1 mediates anxiety-related behavior and hormonal adaptation to stress. Nature Neuroscience 6 (10), 1100–1107.

Nagahara, A.H., Handa, R.J., 1997. Age-related changes in c-fos mRNA induction after open-field exposure in the rat brain. Neurobiology of Aging 18 (1), 45–55.

Naito, H., Inoue, M., Makino, J., 2000. Ultrasonic isolation calls in genetically high- and low-emotional rat pups. Experimental Animals 49 (4), 289–294.

Narrow, W.E., Rae, D.S., Robins, L.N., Regier, D.A., 2002. Revised prevalence estimates of mental disorders in the United States: using a clinical significance criterion to reconcile 2 surveys' estimates. Archives of General Psychiatry 59 (2), 115–123.

Neophytou, S.I., Graham, M., Williams, J., Aspley, S., Marsden, C.A., Beckett, S.R., 2000. Strain differences to the effects of aversive frequency ultrasound on behaviour and brain topography of c-fos expression in the rat. Brain Research 854 (1–2), 158–164.

Ohl, F., Toschi, N., Wigger, A., Henniger, M.S., Landgraf, R., 2001. Dimensions of emotionality in a rat model of innate anxiety. Behavioral Neuroscience 115 (2), 429–436.

Ordyan, N.E., Pivina, S.G., 2004. Characteristics of the behavior and stress-reactivity of the hypophyseal–adrenal system in prenatally stressed rats. Neuroscience and Behavioral Physiology 34 (6), 569–574.

Pacak, K., Palkovits, M., 2001. Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. Endocrine Reviews 22 (4), 502–548.

Palmer, A.A., Printz, M.P., 1999a. Strain differences in Fos expression following airpuff startle in Spontaneously Hypertensive and Wistar Kyoto rats. Neuroscience 89 (3), 965–978.

Palmer, A.A., Printz, M.P., 1999b. Airpuff startle stress elicited fos expression in brain cardiovascular areas of young SHR and WKY rats. Clinical and Experimental Hypertension 21 (7), 1061–1081.

Pezawas, L., Meyer-Lindenberg, A., Drabant, E.M., Verchinski, B.A., Munoz, K.E., Kolachana, B.S., Egan, M.F., Mattay, V.S., Hariri, A.R., Weinberger, D.R., 2005. 5-HTTLPR polymorphism impacts human cingulated-amygdala interactions: a genetic susceptibility mechanism for depression. Nature Neuroscience 8 (6), 828–834.

Phan, K.L., Wager, T., Taylor, S.F., Liberzon, I., 2002. Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. Neuroimage 16 (2), 331–348.

Ploghaus, A., Tracey, I., Gati, J.S., Clare, S., Menon, R.S., Matthews, P.M., Rawlins, J.N., 1999. Dissociating pain from its anticipation in the human brain. Science 284 (5422), 1979–1981.

Poremba, A., Jones, D., Gonzalez-Lima, F., 1998. Classical conditioning modifies cytochrome oxidase activity in the auditory system. European Journal of Neuroscience 10 (10), 3035–3043.

Pratt, J.A., 1992. The neuroanatomical basis of anxiety. Pharmacology & Therapeutics 55 (2), 149–181.

Pryce, C.R., Ruedi-Bettschen, D., Dettling, A.C., Weston, A., Russig, H., Ferger, B., Feldon, J., 2005. Long-term effects of early life environmental manipulations in rodents and primates: potential animal models in depression research. Neuroscience and Biobehavioral Reviews 29 (4–5), 649–674.

Ramos, A., Mormede, P., 1998. Stress and emotionality: a multidimensional and genetic approach. Neuroscience and Biobehavioral Reviews 22 (1), 33–57.

Ramos, A., Correia, E.C., Izidio, G.S., Bruske, G.R., 2003. Genetic selection of two new rat lines displaying different levels of anxiety-related behaviors. Behavior Genetics 33 (6), 657–668.

Rauch, S.L., Savage, C.R., Alpert, N.M., Fischman, A.J., Jenike, M.A., 1997. The functional neuroanatomy of anxiety: a study of three disorders using positron emission tomography and symptom provocation. Biological Psychiatry 42 (6), 446–452.

Ribeiro-Barbosa, E.R., Canteras, N.S., Cezario, A.F., Blanchard, R.J., Blanchard, D.C., 2005. An alternative experimental procedure for studying predator-related defensive responses. Neuroscience and Biobehavioral Reviews.

Rodgers, R.J., Dalvi, A., 1997. Anxiety, defence and the elevated plus-maze. Neuroscience and Biobehavioral Reviews 21 (6), 801–810.

Rodgers, R.J., Johnson, N.J., 1995. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. Pharmacology, Biochemistry and Behavior 52 (2), 297–303.

Rosen, J.B., Schulkin, J., 1998. From normal fear to pathological anxiety. Psychological Review 105 (2), 325–350.

Rosene, D.L., Lister, J.P., Schwagerl, A.L., Tonkiss, J., McCormick, C.M., Galler, J.R., 2004. Prenatal protein malnutrition in rats alters the c-Fos response of neurons in the anterior cingulate and medial prefrontal region to behavioral stress. Nutritional Neuroscience 7 (5–6), 281–289.

Sachdev, R.N., Champney, G.C., Lee, H., Price, R.R., Pickens III, D.R., Morgan, V.L., Stefansic, J.D., Melzer, P., Ebner, F.F., 2003. Experimental model for functional magnetic resonance imaging of somatic sensory cortex in the unanesthetized rat. Neuroimage 19 (3), 742–750.

Sagar, S.M., Sharp, F.R., Curran, T., 1988. Expression of c-fos protein in brain: metabolic mapping at the cellular level. Science 240 (4857), 1328–1331.

Salchner, P., Sartori, S.B., Sinner, C., Wigger, A., Frank, E., Landgraf, R., Singewald, N., 2006. Airjet and FG-7142-induced Fos expression differs in rats selectively bred for high and low anxiety-related behavior. Neuropharmacology, doi:10.1016/j.neuropharm.2006.02.008.

Salome, N., Viltart, O., Darnaudery, M., Salchner, P., Singewald, N., Landgraf, R., Sequeira, H., Wigger, A., 2002. Reliability of high and low anxiety-related behaviour: influence of laboratory environment and multifactorial analysis. Behavioral and Brain Research 136 (1), 227–237.

Salome, N., Salchner, P., Viltart, O., Sequeira, H., Wigger, A., Landgraf, R., Singewald, N., 2004. Neurobiological correlates of high (HAB) versus low anxiety-related behavior (LAB): differential Fos expression in HAB and LAB rats. Biological Psychiatry 55 (7), 715–723.

Sandford, J.J., Argyropoulos, S.V., Nutt, D.J., 2000. The psychobiology of anxiolytic drugs. Part 1: basic neurobiology. Pharmacology & Therapeutics 88 (3), 197–212.

Sandner, G., Oberling, P., Silveira, M.C., Di Scala, G., Rocha, B., Bagri, A., Depoortere, R., 1993. What brain structures are active during emotions? Effects of brain stimulation elicited aversion on c-fos immunoreactivity and behavior. Behavioral and Brain Research 58 (1–2), 9–18.

Sartori, S.B., Ebner, K., Muigg, P., Landgraf, R., Singewald, N., 2005. Differences in substance P neurotransmission between rats with high vs. low trait anxiety/depression. Journal of Neurochemistry 94 (Suppl. 2), 422.

Senba, E., Ueyama, T., 1997. Stress-induced expression of immediate early genes in the brain and peripheral organs of the rat. Neuroscience Research 29 (3), 183–207.

Sewards, T.V., Sewards, M.A., 2002. Fear and power-dominance drive motivation: neural representations and pathways mediating sensory and mnemonic inputs, and outputs to premotor structures. Neuroscience and Biobehavioral Reviews 26 (5), 553–579.

Shah, A.A., Treit, D., 2003. Excitotoxic lesions of the medial prefrontal cortex attenuate fear responses in the elevated-plus maze, social interaction and shock probe burying tests. Brain Research 969 (1–2), 183–194.

Sheehan, T.P., Chambers, R.A., Russell, D.S., 2004. Regulation of affect by the lateral septum: implications for neuropsychiatry. Brain Research and Brain Research Review 46 (1), 71–117.

Shekhar, A., McCann, U.D., Meaney, M.J., Blanchard, D.C., Davis, M., Frey, K.A., Liberzon, I., Overall, K.L., Shear, M.K., Tecott, L.H., Winsky, L., 2001. Summary of a National Institute of Mental Health workshop: developing animal models of anxiety disorders. Psychopharmacology (Berl) 157 (4), 327–339.

Sheng, M., Greenberg, M.E., 1990. The regulation and function of c-fos and other immediate early genes in the nervous system. Neuron 4 (4), 477–485.

Silveira, M.C., Sandner, G., Graeff, F.G., 1993. Induction of Fos immunoreactivity in the brain by exposure to the elevated plus-maze. Behavioral and Brain Research 56 (1), 115–118.

Silveira, M.C., Zangrossi, H., de Barros Viana, M., Silveira, R., Graeff, F.G., 2001. Differential expression of Fos protein in the rat brain induced by performance of avoidance or escape in the elevated T-maze. Behavioral and Brain Research 126 (1–2), 13–21.

Singewald, N., Sharp, T., 2000. Neuroanatomical targets of anxiogenic drugs in the hindbrain as revealed by Fos immunocytochemistry. Neuroscience 98 (4), 759–770.

Singewald, N., Salchner, P., Sharp, T., 2003. Induction of c-Fos expression in specific areas of the fear circuitry in rat forebrain by anxiogenic drugs. Biological Psychiatry 53 (4), 275–283.

Singewald, N., Sinner, C., Hetzenauer, A., Sartori, S.B., Murck, H., 2004. Magnesium-deficient diet alters depression- and anxiety-related behavior in mice—influence of desipramine and *Hypericum perforatum* extract. Neuropharmacology 47 (8), 1189–1197.

Singewald, N., Nguyen, N.K., Hetzenauer, A., Thoeringer, C.K., Wurst, W., Deussing, J., Müller, M.B., Holsboer, F., Keck, M.E., 2005. Conditional CRH1 receptor knockout mice show altered neuronal activation pattern to mild anxiogenic challenge. Journal of Neurochemistry 94 (Suppl. 2), p230.

Smith, M.A., Kim, S.Y., van Oers, H.J., Levine, S., 1997. Maternal deprivation and stress induce immediate early genes in the infant rat brain. Endocrinology 138 (11), 4622–4628.

Sokoloff, L., 1999. Energetics of functional activation in neural tissues. Neurochemical Research 24 (2), 321–329.

Sotres-Bayon, F., Bush, D.E., LeDoux, J.E., 2004. Emotional perseveration: an update on prefrontal-amygdala interactions in fear extinction. Learning & Memory 11 (5), 525–535.

Steimer, T., Driscoll, P., 2003. Divergent stress responses and coping styles in psychogenetically selected Roman high-(RHA) and low-(RLA) avoidance rats: behavioural, neuroendocrine and developmental aspects. Stress 6 (2), 87–100.

Steimer, T., Driscoll, P., 2005. Inter-individual vs line/strain differences in psychogenetically selected Roman high-(RHA) and low-(RLA) Avoidance rats: neuroendocrine and behavioural aspects. Neuroscience and Biobehavioral Reviews 29 (1), 99–112.

Strekalova, T., Spanagel, R., Dolgov, O., Bartsch, D., 2005. Stress-induced hyperlocomotion as a confounding factor in anxiety and depression models in mice. Behavioral Pharmacology 16 (3), 171–180.

Sudakov, S.K., Medvedeva, O.F., Rusakova, I.V., Terebilina, N.N., Goldberg, S.R., 2001. Differences in genetic predisposition to high anxiety in two inbred rat strains: role of substance P, diazepam binding inhibitor fragment and neuropeptide Y. Psychopharmacology (Berl) 154 (4), 327–335.

Sullivan, R.M., Gratton, A., 2002. Prefrontal cortical regulation of hypothalamic–pituitary–adrenal function in the rat and implications for psychopathology: side matters. Psychoneuroendocrinology 27 (1–2), 99–114.

Sumner, B.E., Cruise, L.A., Slattery, D.A., Hill, D.R., Shahid, M., Henry, B., 2004. Testing the validity of c-fos expression profiling to aid the therapeutic classification of psychoactive drugs. Psychopharmacology (Berl) 171 (3), 306–321.

Talbot, P.S., 2004. The molecular neuroimaging of anxiety disorders. Current Psychiatry Report 6 (4), 274–279.

Thakker, D.R., Hoyer, D., Cryan, J.F., 2005. Interfering with the brain: use of RNA interference for understanding the pathophysiology of psychiatric and neurological disorders. Pharmacology & Therapeutics.

Thomas, K.M., Drevets, W.C., Dahl, R.E., Ryan, N.D., Birmaher, B., Eccard, C.H., Axelson, D., Whalen, P.J., Casey, B.J., 2001. Amygdala response to fearful faces in anxious and depressed children. Archives of General Psychiatry 58 (11), 1057–1063.

Thompson, M.A., Ginty, D.D., Bonni, A., Greenberg, M.E., 1995. L-type voltage-sensitive Ca^{2+} channel activation regulates c-fos transcription at multiple levels. Journal of Biological Chemistry 270 (9), 4224–4235.

Titze-de-Almeida, R., de Oliveira, C.L., Shida, H.W., Guimaraes, F.S., Del Bel, E.A., 1994. Midazolam and the *N*-methyl-D-aspartate (NMDA) receptor antagonist 2-amino-7-phosphonoheptanoic acid (AP-7) attenuate stress-induced expression of c-fos mRNA in the dentate gyrus. Cellular and Molecular Neurobiology 14 (4), 373–380.

Uchida, S., Kitamoto, A., Umeeda, H., Nakagawa, N., Masushige, S., Kida, S., 2005. Chronic reduction in dietary tryptophan leads to changes in the emotional response to stress in mice. Journal of Nutritional Science and Vitaminology (Tokyo) 51 (3), 175–181.

Vallee, M., Mayo, W., Dellu, F., Le Moal, M., Simon, H., Maccari, S., 1997. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. Journal of Neuroscience 17 (7), 2626–2636.

Van der Werf, Y.D., Witter, M.P., Groenewegen, H.J., 2002. The intralaminar and midline nuclei of the thalamus. Anatomical and functional evidence for participation in processes of arousal and awareness. Brain Research and Brain Research Review 39 (2–3), 107–140.

Vermetten, E., Bremner, J.D., 2002a. Circuits and systems in stress. I. Preclinical studies. Depression and Anxiety 15 (3), 126–147.

Vermetten, E., Bremner, J.D., 2002b. Circuits and systems in stress. II. Applications to neurobiology and treatment in posttraumatic stress disorder. Depression and Anxiety 16 (1), 14–38.

Vouimba, R.M., Garcia, R., Baudry, M., Thompson, R.F., 2000. Potentiation of conditioned freezing following dorsomedial prefrontal cortex lesions does not interfere with fear reduction in mice. Behavioral Neuroscience 114 (4), 720–724.

Walker, D.L., Toufexis, D.J., Davis, M., 2003. Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. European Journal of Pharmacology 463 (1–3), 199–216.

Wallace, K.J., Rosen, J.B., 2001. Neurotoxic lesions of the lateral nucleus of the amygdala decrease conditioned fear but not unconditioned fear of a predator odor: comparison with electrolytic lesions. Journal of Neuroscience 21 (10), 3619–3627.

Wiersma, A., Konsman, J.P., Knollema, S., Bohus, B., Koolhaas, J.M., 1998. Differential effects of CRH infusion into the central nucleus of the amygdala in the Roman high-avoidance and low-avoidance rats. Psychoneuroendocrinology 23 (3), 261–274.

Wigger, A., Loerscher, P., Weissenbacher, P., Holsboer, F., Landgraf, R., 2001. Cross-fostering and cross-breeding of HAB and LAB rats: a genetic rat model of anxiety. Behavior Genetics 31 (4), 371–382.

Wigger, A., Neumann, I.D., 1999. Periodic maternal deprivation induces gender-dependent alterations in behavioral and neuroendocrine responses to emotional stress in adult rats. Physiology & Behavior 66 (2), 293–302.

Wigger, A., Sanchez, M.M., Mathys, K.C., Ebner, K., Frank, E., Liu, D., Kresse, A., Neumann, I.D., Holsboer, F., Plotsky, P.M., Landgraf, R., 2004. Alterations in central neuropeptide expression, release, and receptor binding in rats bred for high anxiety: critical role of vasopressin. Neuropsychopharmacology 29 (1), 1–14.

Wigger, M., Druckler, E., Muscheites, J., Stolpe, H.J., Kundt, G., Wacke, R., 2003. Comparison of cyclosporine absorption profiles over a 12-month period in stable pediatric renal transplant recipients. Transplantation Proceedings 35 (4), 1300–1303.

Wislowska-Stanek, A., Zienowicz, M., Lehner, M., Taracha, E., Bidzinski, A., Maciejak, P., Skorzewska, A., Szyndler, J., Plaznik, A., 2005. Buspirone attenuates conditioned fear-induced c-Fos expression in the rat hippocampus. Neuroscience Letters 389 (2), 115–120.

Wong-Riley, M.T., 1989. Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. Trends in Neurosciences 12 (3), 94–101.

Wood, S.J., Toth, M., 2001. Molecular pathways of anxiety revealed by knockout mice. Molecular Neurobiology 23 (2–3), 101–119.

World Health Organization, 1992. The Thenth Revision of the International Classification of Diseases and Related Health Problems (ICD-10). World Health Organization, Geneva.

Wu, J.C., Buchsbaum, M.S., Hershey, T.G., Hazlett, E., Sicotte, N., Johnson, J.C., 1991. PET in generalized anxiety disorder. Biological Psychiatry 29 (12), 1181–1199.

Yadin, E., Thomas, E., Grishkat, H.L., Strickland, C.E., 1993. The role of the lateral septum in anxiolysis. Physiology & Behavior 53 (6), 1077–1083.

Yilmazer-Hanke, D.M., Faber-Zuschratter, H., Linke, R., Schwegler, H., 2002. Contribution of amygdala neurons containing peptides and calcium-binding proteins to fear-potentiated startle and exploration-related anxiety in inbred Roman high- and low-avoidance rats. European Journal of Neuroscience 15 (7), 1206–1218.

Zangenehpour, S., Chaudhuri, A., 2002. Differential induction and decay curves of c-fos and zif268 revealed through dual activity maps. Brain Research and Molecular Brain Research 109 (1–2), 221–225.

Zimmerberg, B., Brunelli, S.A., Fluty, A.J., Frye, C.A., 2005. Differences in affective behaviors and hippocampal allopregnanolone levels in adult rats of lines selectively bred for infantile vocalizations. Behavioral and Brain Research 159 (2), 301–311.