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

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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.

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

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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; Kaluﬂe 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 clearly 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) (Gotob 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; Key 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, 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.

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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; Zhangenehpour 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 Zip268), 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 Zhangenehpour, 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 an 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
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 relevantpsychopathological 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

### Table 1

<table>
<thead>
<tr>
<th>Model name</th>
<th>Selection criterion</th>
<th>Enhanced anxiety verified in</th>
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<tbody>
<tr>
<td>Rats</td>
<td></td>
<td></td>
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<tr>
<td>Maudsley Reactive vs. Nonreactive lines</td>
<td>OFD</td>
<td>OFD, OF, L/D, staircase test, EPM (inconsistent)</td>
</tr>
<tr>
<td>Roman low vs. high avoidance</td>
<td>Avoidance (shuttle box)</td>
<td>EPM, OF, L/D, L/D-OF</td>
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<tr>
<td>Syracuse low- vs. high avoidance rats</td>
<td>Avoidance (shuttle box)</td>
<td>FPS</td>
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<tr>
<td>Tsukuba high vs. low emotional line</td>
<td>Runway test</td>
<td>Defecation, OF, I-maze</td>
</tr>
<tr>
<td>HAB vs. LAB</td>
<td>EPM</td>
<td>USV (of pups)</td>
</tr>
<tr>
<td>Floripa L vs. Floripa H rat line</td>
<td>Locomotion in the central area of an OF</td>
<td>OF, EPM, L/D</td>
</tr>
<tr>
<td>Infantile high- vs. low USV</td>
<td>USV</td>
<td>USV, EPM (inconsistent)</td>
</tr>
</tbody>
</table>

| Mice       | EPM | EPM, OA, USV, L/D |

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.
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, z2A adrenoceptor, neurokinin 1 receptor, CRF2 receptor, neuropeptide Y, BDNF (conditional), glucocorticoid receptor (conditional knockout), mineralocorticoid 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 (Orydyan 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 recently 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 McNaughton, 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
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. 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 (review, 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

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**Table 2**

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

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>Challenges</th>
<th>OA</th>
<th>OF</th>
<th>SD</th>
<th>AJ</th>
<th>FG</th>
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<td><strong>Cortex</strong></td>
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<td>Cingulate cortex</td>
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<td>Prelimbic cortex</td>
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<td>Infracorticentral thalamic nucleus</td>
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<tr>
<td>LH, lateral hypothalamic area</td>
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<td>HC, hippocampus</td>
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<td>AMY, amygdala</td>
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<tr>
<td>IHb, lateral habenular nucleus</td>
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<tr>
<td>dIPAG, dorsolateral periaqueductal gray; IPB, lateral parabrachial nucleus; LC, locus coeruleus; 3V, 3rd ventricle; 4V, 4th ventricle, AC anterior comissure, OC optic chiasm.</td>
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</table>

† 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.
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 prelubmic cortex are associated with suboptimal responses to stress and anxiogenic-like stimuli (Jinks and McGregor, 1997; Morgan and LeDoux, 1995; Youimbu et al., 2000), reviewed in Sullivan and Gratton (2002, see, however, e.g. Shah and Treit, 2003).

Lesions of the anterior cingulate and prelubmic 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 (Kalish 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 Morlik, 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 McNaughton, 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; Sowards and Sowards, 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 particularly 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 (Andreattini 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 paraventricular 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 heterocigotes 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 α2 subunit (Atp1a2), 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 comparison of Fischer and Lewis rats, revealing differential published). Examples using this approach in rats include

the PAG (Hetzenauer, Sartori, and Singewald, unpublished). 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

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 SSR1 (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 (Mueller 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 neurocircuits 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/biash 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...
Hyperactivation and hypoactivation has been provided in different anxiety disorders, with studies in PTSD, while in other anxiety disorders results were more inconsistent, also revealing hyperactivation, in panic disorder and PTSD (Bremner, 2004; Cannistraro 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 McNaughton, 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.

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References


Regional dissociations within the hippocampus—memory and anxiety. 
Neuroscience and Biobehavioral Reviews 28 (3), 273–283.
mapping of mouse brain activity after extinction of a conditioned 
Battaglia, M., Ogliari, A., 2005. Anxiety and panic: from human studies to 
animal research and back. Neuroscience and Biobehavioral Reviews 29 
(1), 169–179.
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, 
anxiety-like behavior in mice: a review. Behavioral and Brain 
Research 125 (1–2), 141–149.
behaviour: new pieces in the puzzle. Journal of Comparative 
Neurology 401 (4), 429–436.
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 
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.
Bosch, O.J., Meddle, S.L., Beiderbeck, D.I., Douglas, A.J., Neumann, 
I.D., 2005. Brain oxytocin correlates with maternal aggression: link to 
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.
assays for anxiety: a survey in nine mouse strains. Behavioral and 
Brandao, M.L., Borelli, K.G., Nobre, M.J., Santos, J.M., Albrechet- 
Souza, L., Oliveira, A.R., Martinez, R.C., 2005. Gabergic regulation of 
the neural organization of fear in the midbrain tectum. 
Neuroscience and Biobehavioral Reviews.
Broadhurst, P.L., 1960. Applications of biometrical genetics to the 
inheritance of behaviour. In: Eysenck, H.J. (Ed.), Experiments in 
Brunelli, S.A., 2005. Selective breeding for an infant phenotype: rat pup 
Syracuse strains differ in anxiety, not learning ability. Behavior 
Genetics 33 (6), 677–696.
The effects of early rearing environment on the development of 
GABAergic receptor levels and novelty- 
induced fearfulness in the rat. Neuropsychopharmacology 22 (3), 
219–229.


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. Neurosciences and Biobehavioral Reviews 29 (1), 99–112.


