Review

Candidate genes of anxiety-related behavior in HAB/LAB rats and mice: Focus on vasopressin and glyoxalase-I

Rainer Landgraf[a,*], Melanie S. Keßler[a], Mirjam Bunck[a], Chris Murgatroyd[a], Dietmar Spengler[a], Marina Zimbelmann[a], Markus Nußbaumer[a], Ludwig Czibere[a], Christoph W. Turck[a], Nicolas Singewald[b], Dan Rujescu[c], Elisabeth Frank[a]

[a]Max-Planck-Institute of Psychiatry, Munich, Germany
[b]Institute of Pharmacy, University of Innsbruck, Austria
[c]Department of Psychiatry, Ludwig-Maximilians-University, Munich, Germany

Abstract

Two animal models of trait anxiety, HAB/LAB rats and mice, are described, representing inborn extremes in anxiety-related behavior. The comprehensive phenotypical characterization included basal behavioral features, stress-coping strategies and neuroendocrine responses upon stressor exposure with HAB animals being hyper-anxious, preferring passive coping, emitting more stressor-induced ultrasonic vocalization calls and showing typical peculiarities of the hypothalamic–pituitary–adrenocortical axis and line-specific patterns of Fos expression in the brain indicative of differential neuronal activation. In most cases, unselected Wistar rats and CD1 mice, respectively, displayed intermediate behaviors. In both HAB/LAB rats and mice, the behavioral phenotype has been found to be significantly correlated with the expression of the neuropeptide arginine vasopressin (AVP) at the level of the hypothalamic paraventricular nucleus (PVN). Additional receptor antagonist approaches in HABs confirmed that intra-PVN release of AVP is likely to contribute to hyper-anxiety and depression-like behavior.

As shown exemplarily in HAB rats and LAB mice, single nucleotide polymorphisms (SNPs) in regulatory structures of the AVP gene underlie AVP-mediated phenotypic phenomena; in HAB rats, a SNP in the promoter of the AVP gene leads to reduced binding of the transcriptional repressor CBF-A, thus causing AVP overexpression and overrelease. Conversely, in LAB mice, a SNP in the AVP gene seems to cause an amino acid exchange in the signal peptide, presumably leading to a deficit in bioavailable AVP likely to underlie the total hypo-anxiety of LAB mice in combination with signs of central diabetes insipidus. Another feature of LAB mice is overexpression of glyoxalase-I. The functional characterization of this enzyme will determine its involvement in anxiety-related behavior beyond that of a reliable biomarker. The further identification of quantitative trait loci, candidate genes (and their products) and SNPs will not only help to explain inter-individual variation in emotional behavior, but will also reveal novel targets for anxiolytic and antidepressive interventions.

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*Corresponding author. Tel.: +49 89 30622 495; fax: +49 89 30622 569.
E-mail address: landgraf@mpipsykl.mpg.de (R. Landgraf).

1. Introduction

Anxiety-related and depression-like behaviors represent multifactorial phenomena presumed to have a complex inheritance, involving the interaction of multiple genes in combination with epigenetic and environmental factors in a highly complex manner to affect risk (Gispen-de Wied and Jansen, 2002; Finn et al., 2003; Henderson et al., 2004; De Kloet et al., 2005; Van Praag, 2005). We are interested in establishing genetic animal models of anxiety and comorbid depression, sharing endophenotypes with human psychopathology. As discrete phenotypical features, endophenotypes potentially provide an effective approach to identifying the neurobiological and genetic underpinnings of psychiatric diseases and represent tractable entities to model in rodents (Hasler et al., 2004). While a rodent is certainly not a human and temptations to over-anthropomorphize should be avoided, a great portion of what we do understand today regarding anxiety-related behavior including psychopathology and the genes involved has come from studies conducted in these animals. Despite the many genetic similarities, discoveries in mice and rats do not necessarily lead to corresponding insights in humans, but their extensive genetic and neurobiological homologies give rise to a wide variety of behavioral processes that are well conserved between species. Anxiety- and fear-related behavior represents such a well-conserved strategy, as the ability to sense and avoid potential danger before it strikes is an evolutionary key to survival. However, this strategy comes at a price, as it may give rise to psychopathology. This together with the finding that pathological anxiety evolves from normal anxiety-related behavior (Rosen and Schulkin, 1998) illustrates the potential for rodent models to reproduce and mimic distinct aspects of complex behaviors and neuropsychiatric diseases. In other words, behavioral, anatomical and physiological continuities between rodents and humans are considerable, allowing careful extrapolation of emotions in animals, regardless of whether or not, and in what way, they may be consciously experienced. In this context, the implementation of procedures for analyzing mouse and rat behavior are critical for translating the rapid advances in mammalian genomics into insights relevant to psychiatric disease diagnosis and treatment.

2. HAB and LAB rats and mice represent animal models of extremes in trait anxiety

Each animal model has its own advantages and limitations. In many ways, rat and mouse selection models appear to be superior to, for instance, targeted gene knockouts, since an entire array of neurobiological mechanisms and pathways are selected for, instead of just one altered specific gene and gene product, respectively. More and more strategies are aimed at developing new animal models that consider the impact of correlated factors in determining anxious behavior, including underlying signaling systems that work in an integrated fashion. Importantly, selection animal models keep that integration intact. A major issue with most of these models is that the impact of genetic drift is difficult to assess, thus posing interpretative problems (Henderson, 1997).

Starting from outbred stocks (Wistar rats, CD1 mice) as genotype pools from which to select the specific trait of interest, we decided to use bidirectional selective breeding protocols to generate high (HAB) and low (LAB) anxiety rats and mice. According to Falconer and Mackay (1996), the technique of selective breeding enhances the representation (frequency) of genetic material associated with a particular trait such as anxiety, which shifts the animals' phenotype bidirectionally from the population mean. Therefore, the expectation was that HAB animals have more genetic material that promotes the higher level of trait anxiety, whereas LAB animals accumulate less of this genetic material. We are thus exploiting naturally occurring intra-strain DNA variation in combination with a variety of gene expression and phenotyping data, likely to allow for the identification of candidate genes and ultimately the causal polymorphisms giving rise to or at least contributing to trait anxiety.

In accordance with Rosen and Schulkin (1998), we consider anxiety-related and depression-like behaviors with their considerable inter-individual variation in both outbred and inbred strains and lines as phenomena along a continuum from physiology to psychopathology. In our animal models, this continuum culminates in two opposite poles of extreme behavior: HAB versus LAB. Similarly, several authors claim that the clinical distinction between anxiety and depression is artificial, and rather emphasize common biological diatheses underlying them (e.g., Grillon et al., 2005). Wong and Licinio (2001) proposed a continuum model from anxiety syndromes to mild, moderate and severe depression. Accordingly, the issue of comorbidity of anxiety and depression, which often have overlapping symptoms and also vary over time, has important implications for the treatment of these diseases, in that selective serotonin reuptake inhibitors (now most prescribed treatments for anxiety disorders) and neuropeptide antagonists are successful in treating both certain anxiety disorders and depression (Cryan and Holmes, 2005;

Griebel et al., 2005). Tests of "anxiety-related" and "depression-like" behaviors may reflect corresponding endophenotypes although, according to the continuum hypothesis, there is a lack of clear distinction between them in both animal and human models (Berton and Nestler, 2006). Unfortunately, each of these tests comes with limitations and idiosyncrasies and no one test provides the ideal measure of "anxiety" and "depression" components. Therefore, demonstrating that selective breeding toward HAB and LAB produces alterations on a battery of tests comprising various tasks would provide strong support for a more anxiety- and more depression-related phenotype. Compared to the HAB/LAB rat model, the association between anxiety- and depression-related indices in the corresponding mouse model seems to be rather weak and not invariably present, despite correlative evidence (Krömer et al., 2005). It thus remains to be shown, for instance, whether these indices would cosegregate in an F2 panel.

We use our HAB/LAB animal models with an emphasis on inborn, persistent and robust differences in behavior necessary to advance models of anxiety which allow investigation of behavioral, neuroendocrine and genetic correlates of this trait. Most likely, some degree of correlations and associations exists in all animals, but the evolutionary success of different profiles may vary between species. We here examine the hypothesis, with clinical feedback, that anxiety is associated in such a way that altering one trait, i.e. anxiety, incurs differences in other putative components thought to underlie anxiety-related behavior, including the neuroendocrine hypothalamo–pituitary–adrenocortical (HPA) axis. In this context, recently developed sophisticated tools, such as genotyping, microarray and proteomics, are being utilized to identify candidate genes and their products thought to be associated with phenotypic differences.

As mentioned before, a major issue in this context is that substantial differences in allele frequencies of high versus low lines at many loci may in fact be unrelated to the selected trait. Unreplicated lines, in other words, may generate false-positive associations, making replicate lines necessary (Henderson, 1997). In order to minimize false-positive associations due to genetic drift, we run several independent outbred and inbred sublines within a line in parallel, thus representing replicates started at the same time. Furthermore, in addition to Wistar rats, the same selective and bidirectional breeding protocol was used in CD1 mice to aid in testing the same associations in another rodent species.

As emphasized by Tecott (2003), the technology development for rodent behavioral analyses has lagged behind the pace of innovation in mammalian genetics and genomics, thus still being in its infancy. All the tests used in our studies are considered to mirror particular behavioral endophenotypes rather than the full range of symptoms characteristic of common psychiatric disorders. The most frequently employed class of test designs assesses anxiety-related behavior, relying on the genetic predisposition of rats and mice to avoid open and brightly lit spaces—presumably an innate response evolved to minimize the risk of predation. Similarly, many anxiety disorders are typified by a pervasive avoidance of a feared object or situation. Performance in the elevated plus-maze, which has the potential to predict stress coping strategies (Ducottet and Belzung, 2004), represents the main selection criterion for HAB/LAB animals.

The generation of outbred and inbred HAB/LAB Wistar rats has been described in detail elsewhere (Landgraf and Wigger, 2002, 2003). In brief, as revealed by discriminant, principal component and factor analyses, the main phenotypic difference between HAB and LAB animals is clearly related to "anxiety" (Henniger et al., 2000; Ohl et al., 2001; Salomé et al., 2002, 2006). This trait divergence is highly reliable and robust; there is no gender dependency, and behavioral analyses in four different laboratories, using explicitly non-coordinated test procedures, yielded essentially identical results (Salomé et al., 2002; Neumann et al., pers. comm.). HAB animals, in addition to being extremely anxious, prefer passive and depression-like behaviors including increased floating in the forced swim test (Keck et al., 2003) and freezing during social defeat (Frank et al., 2006). Similarly, HAB rats displayed a more passive coping style in the modified hole board test (Ohl et al., 2001) compared to their LAB counterparts. Along the same lines, HABs emitted more ultrasonic vocalization calls as pups separated from their mothers (Wigger et al., 2001) and as adult animals upon social confrontation, associated with a differential pattern of Fos expression (Frank et al., 2006).

Additional experimental evidence indicates that HAB and LAB rats do not only differ in their anxiety- and depression-related behaviors, but also in their stress vulnerability, with the former being more susceptible and vulnerable. HAB animals, for example, responded stronger than LABs to a weak emotional stressor such as exposure to an open arm of the elevated plus-maze. Resembling neuroendocrine responses of psychiatric patients, they secreted more adrenocorticotropin (ACTH) and corticosterone, indicative of a hyper-reactive HPA axis, suggesting an inborn bias for interpreting a priori ambiguous scenarios as potentially threatening and favoring the selective processing of threat cues (Landgraf et al., 1999). While social defeat data confirmed the prediction that heightened trait anxiety would make rats more prone to experience stress, ACTH and corticosterone were secreted to a higher extent in LAB than in HAB rats, suggesting the possibility of a stimulus-dependent dissociation between behavioral and neuroendocrine stress responses (Frank et al., 2006).

Preliminary data indicate that HAB and LAB animals do not only differ in their (unconditioned) trait anxiety, but also in conditioned fear, with HABs showing signs of markedly delayed extinction (Hetzenauer et al., 2005). While the mechanism of this phenomenon has to be elucidated, approaches like this may contribute to further examine distinction versus interaction between (or even

synergism of) genes, pathways and brain areas underlying anxiety and fear (see also Walker et al., 2003; Singewald, 2006; Sotres-Bayon et al., 2006). In contrast to cued fear conditioning, HAB rats showed lower baseline and fear-sensitized acoustic responses than LABs with no difference in freezing during a startle experiment (Yilmazer-Hanke et al., 2004). The reasons for this discrepancy remain to be shown, though a certain dissociation between learned fear and other types of anxiety-related behavior does seem to exist (Aguilar et al., 2002; Fernandez-Teruel et al., 2002).

Fos expression studies were conducted to characterize neuronal activation patterns in HAB and LAB rats both under basal conditions and in response to stressor exposure (Singewald, 2006). Whereas unstressed HAB/LAB animals showed no line difference in neuronal activity in any analyzed area, exposure to an open arm of the plus-maze or an open field situation induced more Fos-labeling in hypothalamic regions of HAB animals, including the hypothalamic paraventricular nucleus (PVN), medial pre-optic area, and anterior and lateral hypothalamic areas, relative to LABs. In contrast, the latter showed enhanced neuronal activity in the cingulate cortex, thalamic areas (paraventricular thalamic nucleus and lateral habenular nucleus), and partially the hippocampal formation (Salomé et al., 2004). These data have recently been confirmed in a social defeat study, where social subordination resulted in a higher activation of the cingulate cortex and thalamic areas in LABs compared to HABs, who had more Fos-labeled cells in hypothalamic areas, the medial preoptic area, periventricular nucleus, central amygdala and, particularly, the medial amygdala. Interestingly, when exposed to novelty as control, HABs compared to LABs showed less activity in the infralimbic cortex and thalamic regions but more Fos-labeled cells in the parvocellular part of the PVN (Frank et al., 2006). In a combined Fos and pharmacologic fMRI study, Kalisch et al. (2004) have recently provided evidence that the dorsomedial prefrontal cortex (including the cingulate cortex) is not only more activated in hypo-anxious LAB rats, but might also serve as a candidate area for mediating differences in sensitivity to anxiolytic drugs.

While previous cross-mating and cross-fostering studies pointed towards a strong genetic predisposition and a rather limited influence of environmental (maternal) factors (Wigger et al., 2001), Neumann et al. (2005a) have recently succeeded in demonstrating that the genetic predisposition to either high or low anxiety determines the direction of long-lasting effects of adverse early life experiences. In more detail, exposure of male HAB and LAB offspring to periodic maternal separation during the first 2 weeks of life exerted opposing effects on adult emotionality and neuroendocrine stress coping. Whereas postnatally stressed adult HAB offspring were found to be less anxious, identically treated LAB rats became more anxious. Moreover, the hyper-responsiveness of the HPA axis to a mild stressor (exposure to the elevated platform; Neumann et al., 2000a), typical of male HAB rats (Landgraf et al., 1999), became blunted after exposure to postnatal stress with the result that ACTH and

corticosterone responses did not differ anymore between adult HABs and LABs (Neumann et al., 2005a). The finding that chronic consequences of early life stress are strongly dependent on the genetically determined stress vulnerability is further supported by prenatal stress experiments performed in HAB and LAB rats (Bosch et al., 2006). Mechanisms possibly underlying the opposite behavioral effects of prenatal stress seem to include altered expression patterns of hypothalamic arginine vasopressin (AVP) and corticotropin releasing hormone (CRH) mRNAs. Whereas, after prenatal stress, AVP expression was found to be upregulated in LAB rats, the elevated expression of CRH found in HAB rats was significantly reduced (Bosch et al., 2006), these differential effects being not due to maternal factors (Neumann et al., 2005a, b). Importantly, a repeated unpredictable stress treatment for 9 days in adulthood was unable to further alter the level of trait anxiety (Neumann et al., 2005a) indicating that preferentially environmental influences early in life may differentially and chronically modulate the behavioral phenotype of HAB and LAB animals, despite their seemingly rigid genetic predisposition. This hypothesis remains to be verified by using more prolonged and different chronic stress paradigms.

Although HAB and LAB rats represent a useful tool for the study of behavioral and neuroendocrine features of trait anxiety and comorbid depression-like behavior and to explore environmental modulation of genetic predisposition, the possibilities for additional genetic approaches are a priori limited in this species. Genetic approaches such as genome engineering currently available in the mouse make this model organism particularly powerful for the functional analysis of candidate genes and their products underlying anxiety- and depression-related behavior (Tarantino and Bucan, 2000). Therefore, in addition to Wistar rats, we generated high (HAB-M) and low (LAB-M) anxiety CD1 mouse lines as models of extremes in trait anxiety. Resembling our rat model, both mouse lines diverged significantly from unselected "normal" anxiety (NAB-M) and cross-mated counterparts, with HAB-Ms spending less than 10% and LAB-Ms more than 50% of the total test time on the open arms of the elevated plus-maze, independent of gender (Krömer et al., 2005). The behavioral divergence between HAB-M and LAB-M lines steadily increased to reach its maximum after approximately nine generations. The breeding protocol continued with strict sibling mating, trying to conserve genetic polymorphisms underlying either high or low trait anxiety.

The behavioral differences were confirmed by testing the three lines in the dark–light avoidance test and their home cage. In the ultrasonic vocalization test, HAB-M pups emitted more calls than LAB-M pups, with intermediate scores in NAB-M pups. This test, which is primarily independent of locomotor activity, was also used for pharmacological validation. In both HAB-M and LAB-M animals, diazepam induced a decrease in ultrasonic vocalization, indicative of an anxiolytic effect (Krömer et al., 2005). Importantly, in both the forced swim and tail

suspension tests, LAB-Ms displayed significantly less immobility time than HAB-M and NAB-M mice, suggesting reduced depression-like behavior (Cryan et al., 2002; Yoshikawa et al., 2002; Cryan and Mombereau, 2004). After including all three mouse lines in corresponding statistical analyses, both indices of depression-like behavior were found to be highly correlated to each other. Despite a significant correlation between anxiety (% time spent on open arms of the plus-maze) and depression (immobility time in the tail suspension test and floating time in the forced swim test) related indices, it remains questionable whether clinically relevant comorbidity is reliably mirrored in this model. Indeed, the correlation appears to be based on the striking non-depressive behavior of LAB-M mice rather than on a robust association between the corresponding indices (Krömer et al., 2005).

Additional behavioral profiling was achieved by performing detailed videotaped analyses of the mouse lines in different tests, yielding a catalog of readily identifiable behaviors, including head dipping and rearing, a particularly important anxiety measure (Henderson et al., 2004). Both head dipping and rearing confirmed the marked difference in anxiety-related behavior between HAB-M and LAB-M mice (Krömer et al., 2005).

Indices reflecting locomotor activity essentially confirm other reports demonstrating lower activity in mice showing high levels of anxiety- and depression-related behavior (Suaudeau et al., 2000; Do-Rego et al., 2002; El Yacoubi et al., 2003) and, vice versa, high locomotor activity in animals displaying low levels of anxiety- and depression-related behavior (Ferguson et al., 2004). Depending on the behavioral test used (Suaudeau et al., 2000), this difference in locomotor activity may or may not explain differences in anxiety-related behavior. It is of note in this context that suppression of locomotor activity is one of the cross-test dimensions of anxiety (Henderson et al., 2004).

3. The neuropeptide vasopressin and its release within distinct areas of the rodent brain

Numerous studies have used animal models to examine candidate genes for involvement in anxiety-related behavior. One promising category of candidates encodes neuropeptides involved in multiple and variable modes of interneuronal signaling (Landgraf and Neumann, 2004). More than any other class of neurochemicals, neuropeptides have been found to be critical for behavioral plasticity, social diversity, emotionality and cognitive abilities. While complexity of a trait probably arises from the numerous ways in which a modest number of genes, alleles and gene products interact as well as from epigenetic and environmental influences, a hypothesis-driven candidate gene approach focuses on a particular gene and its product.

The neuropeptide AVP is not only a major physiological regulator of renal water excretion, acting in the kidney to increase the water permeability of the renal distal tubules and collecting ducts, thereby accelerating water reabsorption. Released within distinct brain areas, it is critically involved in the regulation of anxiety-related and depression-like behaviors (see Sections 4–6).

Centrally released AVP may act as a neurotransmitter and, even more important, as a neuromodulator. Through the latter type of information transfer, the brain is liberated from the constraints of wiring, since neuropeptides can reach any point of target neurons, thus enormously increasing the information handling capacity of neurons. While the distinction between transmitters, modulators and hormones has its heuristic value, explaining high speed, spatial precision and a theoretically unlimited variability in signaling, probably even simple information transfers use a combination of these modes of communication. Coordinated patterns of response to a neuropeptide, in other words, are likely to reflect a coordinated combination from synaptic through non-synaptic to hormonal actions, often in a synergistic manner, suggesting that theoretically contrasting views are practically reconcilable (Landgraf and Neumann, 2004).

Neuropeptides come into play when the brain is trying to adapt to various types of challenges (Gross and Hen, 2004; Landgraf, 2006). The central release of AVP in distinct brain areas is regulated by a wide variety of physiological, pathophysiological and pharmacological stimuli. Forced swimming, for example, is a combined emotional and physical stressor, causing the dendritic release of AVP within the SON and PVN without affecting its systemic secretion. In contrast, plasma levels of oxytocin, a structurally and functionally related neuropeptide, paralleled the changes in dendritic release (Wotjak et al., 1998). The magnocellular neurons are likely to be the predominant source of intra-PVN release of AVP (Wotjak et al., 2001), as dendritic release in the absence of peripheral secretion was followed by increased mRNA levels in magnocellular neurons only, indicating a refill of depleted AVP stores assigned to dendritic release.

The continuum of central AVP release from low basal release via stimulated levels in the extracellular fluid of the brain inducing anxiety- and depression-like behavior up to very high levels explains the wide spectrum of AVP effects in the brain, with very high levels and/or duration finally leading to psychopathology. The continuum of central AVP release thus suggests that its actions upon stressor exposure are initially beneficial to the individual serving to adjust physiology and behavior to increase short-term survival, possibly at the potential expense of increasing susceptibility to disease over the long term (Landgraf, 2006). Psychopathology is thought to include both a genetic predisposition and dysregulated responses to stress (Finn et al., 2003; De Kloet et al., 2005; Van Praag, 2005). The ability of AVP to both mediate genetic polymorphisms (Murgatroyd et al., 2004) and respond to stressful stimuli (Wotjak et al., 1996; Ebner et al., 1999) makes the release of this neuropeptide in distinct brain areas a key process for converging (i.e., genetically and environmentally

driven) behavioral regulation, both under physiological and psychopathological conditions. Accordingly, strong and chronic stimulation of the central AVP system may play a causal role in the etiology and symptomatology of affective disorders (Scott and Dinan, 2002; Ring, 2005; Landgraf, 2006).

4. Clinical and preclinical approaches suggest centrally released vasopressin is critically involved in anxiety- and depression-like behavior

Indeed, almost 30 years ago, Gold et al. (1978) already hypothesized an involvement of endogenous AVP in the pathophysiology of affective disorders, based on evidence from animal studies. Altemus et al. (1992) succeeded in identifying a critical role of AVP in obsessive–compulsive disorder. They reported an elevation of AVP concentrations in both the cerebrospinal fluid (CSF) and plasma of patients who often conduct stereotyped, repetitive behaviors to relieve anxiety. It is noteworthy that stereotyped behavior, such as repetitive grooming, has been proposed as ethological correlate to obsessive–compulsive disorder in rodents (Stein et al., 1992). Indeed, central administration of AVP in hamsters produced intense stereotypic grooming and flank marking behavior, an effect blocked by both V1 receptor antagonists and clinically relevant drugs (Ferris et al., 1988, 2001). Similarly, the stereotypic behavior of scratching and repetitive autogrooming elicited by central administration of AVP was absent in male V1a receptor knockout mice (Bielsky et al., 2004).

Inconsistent associations have been reported between CSF and plasma AVP levels and depression. Brunner et al. (2002) failed to show differences in CSF and plasma AVP levels between drug-free depressed suicide attempters, depressed non-suicidal patients and neurological control subjects. More recently, above-normal plasma AVP values were reported to be linked to a family history of depression and mixed anxiety and retardation (Goekoop et al., 2006), although the functional involvement of plasma AVP remains questionable in this context. De Bellis et al. (1993) measured CSF concentrations of AVP and CRH in depressed patients before and after fluoxetine treatment. Supporting the findings of Altemus et al. (1992), both neuropeptides were found to be reduced by the antidepressant, giving rise to the hypothesis that the clinical efficacy of fluoxetine may be related to diminution of central AVP and CRH.

Evidence has been accumulated in neuroendocrine studies that in depression as well as in chronic stress, there is a gradual shift from CRH- to a more AVP-controlled HPA axis activity (De Goeij et al., 1992; Dinan et al., 1999; Volpi et al., 2004). Accordingly, an involvement of AVP in neuroendocrine correlates of anxiety disorders and comorbid depression is suggested by a variety of animal (Keck et al., 2002; Alonso et al., 2004) and clinical (von Bardeleben et al., 1985; Dinan et al., 1999; Zhou et al., 2001; Scott and Dinan, 2002) studies. Post mortem analyses of the brains of patients with a known history of depression support the concept that endogenous AVP may be involved in the pathophysiology of the disorder. The number of AVP-(and oxytocin- and CRH-) expressing neurons in the PVN was reported to be significantly increased in depression (Raadsheer et al., 1994; Purba et al., 1996), thus resembling the situation in HAB rats (Keck et al., 2003; Wigger et al., 2004) and in a model of stress-induced depression (Nakase et al., 1998). Despite neuropeptide overexpression, reflecting signs of neuronal activation, the total number of PVN neurons was found to be reduced by approximately 50% in patients suffering from major depression and bipolar disorder, with no change in the supraoptic nucleus. This phenomenon is probably due to a selective loss of interneurons in inhibitory circuits of the PVN (Manaye et al., 2005).

Excessive anxiety and social avoidance are common features of anxiety disorders. Interestingly, polymorphisms in both the AVP gene promoter (Murgatroyd et al., 2004) and the V1a receptor gene (Hammock et al., 2005) generate interindividual differences in anxiety and socio-behavioral traits. Increasing evidence indicates an influence of the AVP system on emotionality and social behavior in humans (Young, 2001; Young et al., 2002; Storm and Tecott, 2005). For instance, AVP self-administered by means of an intranasal spray may influence aggression and anxiety by biasing individuals to respond to emotionally ambiguous social stimuli as if they were threatening and aggressive (Thompson et al., 2004, 2006). Related facial responses and vocalization are critical components for the social engagement system in which endogenous AVP is thought to be involved (Porges, 2001). Another paper (Fries et al., 2005; see also Carter, 2005) suggested an effect of social deprivation on urine AVP and oxytocin by comparing family- versus orphan-reared children. While both neuropeptide systems appeared to be affected by early social experience, the causal relationship between urine neuropeptides on the one hand and centrally released neuropeptides on the other remains questionable. Apart from independent central and peripheral AVP release patterns and an effective blood–brain barrier separating the central and peripheral compartments for endogenous neuropeptides, responses of at least plasma and urine AVP to a variety of stimuli have to be carefully examined before final conclusions can be drawn.

In addition to AVP, its receptors are of growing interest for psychiatric research. Based on vole data (Young et al., 1999), the V1a receptor gene was investigated as a reasonable candidate for autism susceptibility genes and for social contact. While differences in V1a receptors at the amino acid level are unlikely to confer genetic variability to autism, variability outside the coding sequence might be involved (Kim et al., 2002; Young et al., 2002; Wassink et al., 2004; Yirmiya et al., 2006). Furthermore, AVP V1a receptor gene polymorphisms seem to be associated with creative human dancing which can be understood as a form of social communication sharing an evolutionary history

with mating displays and affiliative behavior observed across the vertebrates (Bachner-Melman et al., 2005). Similarly, resembling findings in rodents (Griebel et al., 2002, 2005), the AVP V1b receptor seems to play a role in depressed patients. Indeed, genetic variations in the V1b gene could be associated with increased susceptibility to affective disorders, a SNP haplotype protecting against recurrent major depression (Van West et al., 2004).

Clinically oriented studies are flanked by attempts to establish a role for centrally released AVP in animals, particularly rodents. In rats, AVP fulfills the important criterion of being released upon anxiety-provoking stimulation in both hypothalamic and limbic brain areas (Wotjak et al., 1996; Ebner et al., 1999). In the mid-1990s, antisense targeting and antagonist approaches (Landgraf et al., 1995; Liebsch et al., 1996) provided the first hints indicating that centrally released AVP interacting with its V1a receptor subtype might act as an endogenous anxiogenic neuropeptide in the rat. Since then, this hypothesis has been confirmed in multiple approaches and species (for review, see Landgraf, 2006). While there is a lack of an anxiety-related phenotype in female V1a (Bielsky et al., 2005a) and male V1b (Egashira et al., 2005) receptor null mutants, male mice lacking the functional V1a receptor exhibited reduced anxiety-related behavior (Bielsky et al., 2004), and a critical involvement of V1b receptors in the lateral septum of the rat brain is indicated by antidepressive-like effects of the non-peptide V1b antagonist SSR149415 (Stemmelin et al., 2005). Similar to fluoxetine, SSR149415 reversed the stress-induced suppression of neurogenesis in a mouse model of depression, partly explained by a reduction of stress-induced hyper-activity of the HPA axis (Alonso et al., 2004). This finding, together with the mitigating effect of paroxetine on AVP overexpression in the PVN of HAB rats (Keck et al., 2003), supports the concept that both behavioral and neuroendocrine effects of selective serotonin reuptake inhibitors might, at least partially, be mediated by central AVP (see also De Bellis et al., 1993). Conversely, upregulation of the V1a receptor in the ventral pallidum of prairie voles (Pitkow et al., 2001) and in the lateral septum of mice (Bielsky et al., 2005b) caused increased levels of anxiety, thus confirming a role of centrally released AVP as an endogenous anxiogenic neuropeptide.

Further support for a critical role of the AVP system in anxiety- and depression-related behavior is provided by HAB and LAB rats and mice, as described in the following sections.

5. A regulatory SNP in the vasopressin gene promoter causes vasopressin overexpression and probably heightened anxiety and depression-like behavior in HAB rats

Based on behavioral and neuroendocrine phenotyping of HAB and LAB animals (see Section 2) and data

suggesting a role of centrally released AVP in anxiety and depression (see Section 4), the AVP gene was considered a candidate gene of trait anxiety and, possibly, comorbid depression. Indeed, resembling anxious Roman low avoidance rats (Aubry et al., 1995), HAB rats expressed more AVP at the level of the hypothalamic PVN than their LAB counterparts, both under basal conditions and upon stressor exposure (Keck et al., 2003; Wigger et al., 2004; Bosch et al., 2006). As demonstrated by immuno-histo-chemistry and in vivo microdialysis, the increased synthesis is accompanied by elevated (i) AVP contents in both parvo- and magnocellular subdivisions of the PVN, this content being correlated with individual anxiety-related scores (% time spent on open arms of the elevated plus-maze) (Murgatroyd et al., 2004), and (ii) intra-PVN release of AVP in male HAB relative to LAB animals (Wigger et al., 2004). The specificity of this phenomenon is underlined by the finding that the concentration of oxytocin measured in the same dialysate samples did not differ between the lines. In contrast, in female HAB rats, the increased central release of oxytocin correlates with signs of enhanced offensive behavior displayed in the maternal defense test, linking aggression to anxiety (Bosch et al., 2005).

To examine the behavioral significance of AVP over-expression and release, inverse microdialysis was used to administer a V1 receptor antagonist bilaterally into the PVN of freely behaving male HABs. This approach provided the advantage of avoiding an acute (and possibly more stressful) injection and of continuously delivering an efficacious amount of the antagonist, thus blocking the effects of locally released AVP. Compared to controls, antagonist-treated HABs tended to be less anxious and showed reduced depression-like behavior (Wigger et al., 2004). Additionally, repetitive intra-PVN administration of a selective AVP gene antisense oligodeoxynucleotide sequence was found to reduce both anxiety and depression-like behaviors of these animals (Frank, unpublished observation).

In addition to behavioral consequences, AVP over-expression in HABs is likely to promote HPA axis hyper-reactivity to stressors (Landgraf et al., 1999). Furthermore, the pathological outcome of the combined dexamethasone suppression/CRH challenge test found in HAB rats could be brought back to normal levels by intravenous administration of a selective V1 receptor antagonist (Keck et al., 2002). Remarkably, long-term treatment with the antidepressant drug paroxetine did not only result in a reduction of depression-like behavior and a normalization of the dexamethasone/CRH test, but also in a normalization of AVP overexpression in the PVN (Keck et al., 2003). These effects, which were all absent in LAB controls, highlight the critical involvement of AVP in indices of behavioral and neuroendocrine peculiarities in HAB rats, including clinical extrapolations.

The next step was to examine whether the HAB-specific allele of the AVP gene promoter shows any sequence variations compared to that of the wild type. Indeed, sequence analysis of the AVP locus revealed 10 single nucleotide polymorphisms (SNPs) within a 2.7 kb region of the AVP promoter sequence differing between the HAB and LAB animals. Additionally, a single base pair substitution occurred in the first intron (T(+549)C) of the AVP gene itself. HAB rats were uniformly homozygous for all of these SNPs while LAB animals, in turn, were homozygous for the published wild type nucleotide sequence. Therefore, allelic differences at the AVP locus most likely relate to the existence of different haplotype blocks. To determine the incidence of the corresponding alleles, we examined 100 Wistar rats, purchased from Charles River, for the absence or presence of these polymorphisms. Three animals were found to be heterozygous indicating a gene frequency of 1.5% for the HAB allele in the outbred Wistar rat population. As mentioned before, selective breeding enriches for genetic information associated with a particular trait, which shifts the animals' phenotype from the population mean (Falconer and Mackay, 1996). During the decades of selective and bidirectional breeding, we probably selected (without knowing it at that time) those animals who carried the HAB allele with strict trait selection leading to homozygosity at loci conferring trait anxiety and comorbid depression-like behavior. Therefore, our data strongly suggest that the bidirectional inbreeding protocol selected for this HAB allele which, in turn, might critically contribute to increased trait anxiety in this line. In accordance with this view, all three heterozygous rats showed a 2-fold higher anxiety-related behavior on the elevated plus-maze than the Wistar population mean (7.2% versus 13.6%; Murgatroyd et al., 2004).

Among the SNPs localized in the AVP gene promoter, the SNP A(-1276)G seemed to be of particular interest, as it is embedded in a potential transcription factor DNA-binding site known as CArG box. This cis-regulatory motif is well known to be bound by the transcriptional repressor CBF-A (Kamada and Miwa, 1992). Then, does CBF-A consequently discriminate between binding to the HAB versus LAB CArG boxes? In fact, for different concentrations of CBF-A, binding to the CArG box derived from the HAB allele was diminished when compared to the LAB allele. Competition assays substantiated this finding by showing that an oligonucleotide corresponding to the HAB sequence was clearly less efficient than the one derived from the LAB sequence in competing for CBF-A binding. To test whether these differences measured by in vitro DNA-binding assays actually correlate with transcriptional behavior, reporter gene assays were used. Coexpression of increasing concentrations of CBF-A repressed AVP promoter activity in two different cell lines, with the HAB AVP sequence being less prone to repression than the LAB construct at similar CBF-A concentrations. This finding begged the question of whether differential regulation of HAB and LAB promoter constructs is confined to the intact AVP promoter and thus possibly reflects a context-specific regulatory behavior of CBF-A as has been frequently observed in the case of transcriptional repression. On the other hand, the CBF-A site might encode all the necessary information to endow CBF-A with a role as repressor and, if so, it may confer repression to a heterologous promoter. To distinguish between these two possibilities, increasing amounts of CBF-A were cotransfected with a minimal promoter construct harboring three copies of either the LAB or HAB allele derived CArG boxes. We observed, at the highest doses of CBF-A, a striking 4-fold repression of the LAB line derived CArG box when compared to the HAB (Murgatroyd et al., 2004). These findings enforce the relevance of in vitro DNA-binding assays to predict transcriptional activity of the AVP promoter and, furthermore, show this regulatory SNP to operate autonomously in conferring different degrees of transcriptional repression. Conceptually, these findings demonstrate that impaired CBF-A binding to the HAB sequence, due to SNP A(-1276)G, results in a weakened transcriptional repression of the HAB allele by CBF-A, thus potentially contributing to AVP overexpression.

So far, the results support a role of CBF-A in repression of the AVP gene promoter in vitro and suggest a similar role in vivo. To evaluate this hypothesis in more detail, we examined whether and to which extent CBF-A colocalizes with AVP. Double immunofluorescence experiments for CBF-A and AVP expression evidenced broadly overlapping AVP and CBF-A expression in both the magnocellular and parvocellular subdivisions of the PVN. Interestingly, in situ hybridization and immunohistochemistry data did not reveal any differences in CBF-A expression and content in the PVN between HAB and LAB rats. This rules out the possibility that lower levels of CBF-A per se could underlie the increased AVP expression present in the HAB AVP neurons, further strengthening the importance of the cis-regulatory CArG polymorphism (Murgatroyd et al., 2004).

The line-specific differences in the sequence of the AVP promoter suggest a causal role in the differential AVP expression. If so, HAB and LAB lines would represent distinct disease entities with the AVP gene behaving as a vulnerability gene solely in the former. Yet, on the contrary, the intra-PVN overexpression of AVP in HAB rats could be unrelated to their polymorphic promoter structure, but instead primarily result from a shift in synaptic input, providing more excitation and/or less inhibition. To examine this critical issue, we cross-mated homozygous HAB and LAB rats to produce heterozygous animals with both promoter alleles situated in the same cellular environment, i.e. receiving the same synaptic input. Importantly, these cross-mated animals showed a strictly intermediate behavioral phenotype. PVNs from these animals were then isolated to measure allele-specific transcription rates of AVP and, additionally, of oxytocin which served as a control in these experiments. In more

detail, by extracting RNA from the PVN and cloning the reverse-transcribed specific cDNAs, it was possible to track precisely which RNA species is more prevalent in vivo, given that the transcriptional activity of the corresponding promoter allele critically depends on the presence or absence of regulatory polymorphisms in the promoter structure. To do so, we took advantage of the fact that the SNP in the AVP gene itself allowed the determination of the origin of RNA species as being derived from either the HAB or LAB allele. Indeed, the allele-specific AVP transcription rates showed a significant difference between the two alleles with the HAB-specific one displaying a 50% increase. This difference could not be due to different cellular environments, including synaptic input. No difference was found for the oxytocin gene which localizes 11 kb away from the AVP locus, i.e. HAB and LAB oxytocin alleles contributed equally to the RNA pool (Murgatroyd et al., 2004). These allele-specific transcription data corroborate previous in situ hybridization and microdialysis in vivo data (Wigger et al., 2004), all showing significant line differences in AVP rather than oxytocin, at least in male animals.

Why is AVP overexpression restricted to the hypothalamic PVN but puzzlingly absent in the SON, despite the presence of the same promoter polymorphisms and of similar levels of CBF-A mRNA and protein in both nuclei? According to Murgatroyd et al. (2004), nucleus-specific posttranslational modifications of CBF-A are possible, resulting in different isoforms likely to display differential activity irrelevant of its similar expression in the PVN and SON. Moreover, multi-layered interactions between *cis*-regulatory promoter sequences and the cell-type specific pool of available transactivators direct tissue-specific expression and regulation of any gene. In this view, the identified AVP polymorphism and CBF-A appear necessary but not sufficient to confer elevated AVP expression. Indeed, they appear embedded in a functionally linked entity, which embraces tissue-specific expression on the one hand, and functional specificity on the other hand. We propose that the AVP CArG-box polymporphism depends on a permissive environment, of which CBF-A expression is one critical variable, to endow this site with functional activity. We furthermore reason that this seemingly paradoxical behavior of AVP expression at instead strongly attests to the specificity and validity of a genetic mechanism contributing to a complex disease. It is of note in this context that the PVN, in contrast to the SON, has been described to be causally involved in depression (Manaye et al., 2005). In general, these findings exemplify how a universal mechanism, i.e. DNA variation, is critically controlled at the level of tissue specificity, which in turn determines the functional and pathological outcome.

Inter-individual phenotypic variation is central to evolutionary adaptations underlying natural and artificial selection and also determines individual susceptibility to common diseases including psychiatric disorders. Phenotypic diversity is characterized by differences in both degree (primarily attributable to *cis*- and *trans*-acting sequence variants that affect gene regulation) and kind (attributable to non-synonymous changes). In accordance with this view, functional promoter element SNPs, being of roughly equal priority for genotyping in complex diseases as non-synonymous changes, contribute substantially to common phenotypes (Buckland et al., 2004). HAB rats lack functionally relevant changes in the coding part of the AVP gene, but instead are homozygous for the polymorphic promoter region. Therefore, the HAB-specific AVP promoter represents a natural model for AVP overexpression and highlights in turn cognate molecular pathways which potentially fuel the resulting inter-individual variation in anxiety- and depression-related behaviors along a continuum that leads to psychopathology.

While our findings highlight combined phenotypic and molecular-genetic analyses of candidate genes and their products as a promising tool to uncover the basis of anxiety-related behavior, they do not indicate centrally released AVP as the sole, but certainly as an important candidate. Our attempts to conserve the polymorphisms underlying the genetic predisposition to either hyper- or hypo-anxiety by strict sibling mating and, at the same time, to minimize the problem of genetic drift by running parallel, independent sublines in a given line and replicating key phenomena in another species do not necessarily exclude (slight) phenotypic shifts from occurring over many generations (see also El Yacoubi et al., 2003). CRH mRNA expression at the level of the hypothalamic PVN was described to be slightly higher in HAB than LAB rats ($p = 0.12$; Wigger et al., 2004). Five to six generations later, this difference reached statistical significance, as shown independently in several laboratories (Bosch et al., 2006; Márquez, Nadal and Armario, University of Barcelona; Sartori and Singewald, University of Innsbruck; unpubl. observations). There are CRH-containing pathways from the central nucleus of the amygdala via the bed nucleus of the stria terminalis (BNST) to the PVN, with the BNST playing a special role in anxiety unrelated to immediate environmental threats (Walker et al., 2003). Since these pathways could regulate CRH synthesis in the PVN (Champagne et al., 1998), the decreased CRH mRNA expression within the BNST of HABs (Wigger et al., 2004) could have additional influence on their PVN. Because not only CRH mRNA expression, but also CRH receptor 2 binding was shown to be elevated in the PVN of HAB rats (Wigger et al., 2004), this neuropeptidergic system and its contribution to the behavioral and neuroendocrine phenotype of HAB/LAB animals will attract more attention in the future. Based on such findings, both AVP and CRH could play an important role towards an integration of neurobiology, neuroendocrinology and psychiatry.

6. The role of vasopressin and glyoxalase-I in selectively bred mice

Preliminary data indicate that the intra-PVN expression of AVP is significantly lower in LAB-M than HAB-M and

unselected CD1 (NAB-M) mice, thus resembling HAB/ LAB rats. Furthermore, again resembling corresponding data in rats, individual pairs of data obtained from HAB-M, NAB-M and LAB-M mice suggest a positive correlation between anxiety-related behavior on the elevated plus-maze and AVP expression at the level of the PVN. In light of the common dilemma when trying to associate the expression of a single gene with complex behaviors, this result further substantiates a conceptual link between AVP expression and the phenotype associated with anxiety. Correlative evidence, however, does not necessarily indicate a reliable association. Thus, additional studies, including antagonist and F2 panel approaches, have to further examine both the strength and the functional impact of this putative association.

The hypothesis that the total non-anxiety of LAB-M mice, apparently caused by a deficit in AVP synthesis, processing and/or trafficking, might be accompanied by deficits in the peripheral AVP system, was tested in control and water-deprived animals. Indeed, LAB-M mice show signs of central diabetes insipidus, including excessive drinking and the inability to properly retain body water. Preliminary data indicate that a non-synonymous SNP, resulting in an amino acid exchange in the signal peptide (Murgatroyd, Breske and Spengler, unpublished observation), might cause the AVP deficit with functional consequences for both anxiety-related behavior (total non-anxiety) and water balance (signs of central diabetes insipidus). Remarkably, both AVP-deficient diabetes insipidus rats (Herman et al., 1986) and patients (Bruins et al., 2006) were described to show signs of reduced anxiety.

The apparently similar involvement of centrally released AVP in rats and mice is noteworthy as both species differ markedly in neurohypophysial peptide concentrations. This is indicated by much higher basal concentrations of AVP and the structurally and functionally related neuropeptide oxytocin in plasma of mice compared to rats (approx. 100 fold; Engelmann and Landgraf, unpubl. observation). Although plasma concentrations do not necessarily reflect central release patterns, species differences in central AVP release that is largely unknown in the mouse seem to be likely as well.

Another feature typical of mice is their anxiety level-dependent expression of glyoxalase-I (Krömer et al., 2005). This cytosolic enzyme, which attracts increasing attention for its role in psychopathology (Chen et al., 2004), is present in all cells and tissues of the organism. It has been suggested to play a major role in the detoxification of dicarbonyl metabolites, mostly methylglyoxal and glyoxal which represent potent cytotoxic metabolites. Glyoxalase-I-related metabolism is thus linked to countering dicarbonyl stress and associated protein damage by dicarbonyl glycation (Thornalley, 2006). Although the enzyme is certainly not one of the usual suspects in signal transduction of emotionality, a possible connection between glyoxalase-I and depression was found in a linkage study

of families with depressive disease. Subgroups with unipolar affective disease showed evidence for an association with the glyoxalase-I locus; unfortunately, the level of anxiety was not scored in this study (Tanna et al., 1989).

In both brain tissue and blood, HAB-Ms were recently reported to underexpress and LAB-Ms to overexpress the enzyme relative to unselected NAB-M and cross-mated animals. As determined by proteomic analyses, this gender-independent expression pattern is so robust that it may serve as a biomarker of anxiety-related behavior in the HAB-M/ LAB-M model, helping to categorize subsets of animals in a more reliable and consistent manner (Krömer et al., 2005). The validity of glyoxalase-I as a protein marker of anxiety-related behavior has further been confirmed by (i) microarray experiments revealing that the enzyme exhibits differential expression in the PVN, with LAB-M mice expressing twice as much RNA under basal conditions than HAB-Ms, (ii) additional testing of inbred mouse strains differing in their trait anxiety, (iii) the unambiguous identification of the HAB-M versus LAB-M phenotype in blood samples in a blind manner (Krömer et al., 2005), and (iv) reduced levels in the blood of patients suffering from anxiety disorders as compared to levels measured in healthy control subjects (Ditzen et al., 2006). Contradictory results were recently reported by Hovatta et al. (2005) describing a decreased anxiety-related behavior upon inhibition of glyoxalase-I expression by RNA interference. A possible contribution of increased cellular proteolysis to this behavioral response (Thornalley, 2006) remains to be shown. Thus, we actually do not know how a ubiquitous metabolic enzyme might be involved in anxiety and depression; the hypothesis of metabolic depression in major depression (Tsiouris, 2005) seems to be of interest in this context.

The predictive validity of glyoxalase-I to identify different levels of anxiety-related behavior provides the basis for future testing, including its impact beyond that of a biomarker. Furthermore, sequence analysis of the regulatory regions of the glyoxalase-I gene will be pertinent to characterize the mechanisms underlying its differential expression.

7. Perspective

Candidate gene approaches in combination with gene expression profiling and proteomics as described here are expected to complement other methods including quantitative trait loci mapping in rats and mice (Fernandez-Teruel et al., 2002; Henderson et al., 2004; Flint et al., 2005). The newly identified genes and their products should further our understanding of mechanisms and circuits that are critical for the regulation of normal and pathological anxiety-related behavior in both rodents and humans.

As particularly shown in HAB rats and LAB-M mice, SNPs in the regulatory structures of the AVP gene may cause either over- or underexpression of AVP in the hypothalamic PVN, thus probably contributing to hyper-anxiety and hypo-anxiety, respectively, with the latter

occurring in combination with signs of central diabetes insipidus. Our understanding of the role of centrally released AVP in anxiogenesis and psychopathology is based on the neurobiology and physiology of this neuropeptidergic system. Its evolution and involvement in different signaling pathways provided the basis for the evolution of multiple body and brain functions and, vice versa, the latter exerted selection pressure for the further development of neuropeptidergic circuits. Thus, AVP circuits in distinct brain areas, novel emotional facets and individual emotional profiles probably co-evolved. It is easy to envisage the value that such processes could have for most animal species and the selection pressure favoring their evolution. In fact, there is virtually no behavioral facet unrelated to central AVP. Additionally the potential for synergistic effects is given by the involvement of this neuropeptide in neuroendocrine stress regulation (HPA axis), homeostasis etc. as well as its interaction with other neuropeptides, presumably balancing and adjusting biologically adequate behavior in a fine-tuned manner. Among them, two neuropeptides seem to be of particular interest: CRH, likely to complement the anxiogenic effects of central AVP (Dunn and Berridge, 1990; Arborelius et al., 1999), and oxytocin, closely related to AVP in many aspects, but acting as an anxiolytic neuropeptide (Neumann et al., 2000b; Bosch et al., 2005; Ring et al., 2006; Windle et al., 2006). Thus, while AVP and CRH could represent Scylla and Charybdis in behavioral regulation, oxytocin, like Circe, would provide anxiolytic and calming influences, beneficial to relief, love and reproduction. These and other possible interactions among centrally released neuropeptides in shaping anxiety-related behavior (e.g., Saavedra and Pavel, 2005) will be addressed in future animal and human studies.

The pleiotropic effects of AVP are based on its multiple and variable release patterns in distinct brain areas and subsequent receptor-mediated modes of interneuronal communication. Reflecting release patterns along a continuum, these effects may be initially beneficial to the individual serving to adjust behavior and physiology to increase short-term survival, possibly at the potential expense of increasing susceptibility to disease over the long term if release overrides certain set-points in duration and/or extent. Thus, the key role of AVP in behavioral, neuroendocrine and physiological regulation makes this system particularly vulnerable to genetic polymorphisms that lead to altered neuropeptide expression and, consequently, far-reaching functional consequences, including psychopathology. This, together with genetic variability of the AVP receptors (Bielsky et al., 2005b; Hammock et al., 2005; Hammock and Young, 2005), may at least partly explain inter-species differences and inter-individual variation in emotional (and other) behaviors, making the AVP system not only a critical substrate for the evolution of emotional behavior, but also a promising target for therapeutic interventions.

Notably, many recently identified candidate genes of anxiety encode enzymes linking metabolic pathways with anxiety-related behavior. In this context, the characterization of functional SNPs in the glyoxalase-I gene and of the mechanisms underlying its anxiety-dependent expression will be as essential as the identification of other quantitative trait loci, candidate genes and SNPs likely to contribute to phenotypic variation and psychopathology.

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