Protein Biomarkers in a Mouse Model of Extremes in Trait Anxiety*

Claudia Ditzen, Archana M. Jastorff, Melanie S. Kessler, Mirjam Bunck, Larysa Teplytska, Angelika Erhardt, Simone A. Krömer, Jeeva Varadarajulu, Bianca-Sabrina Targosz, Eser F. Sayan-Ayata, Florian Holsboer, Rainer Landgraf, and Christoph W. Turck‡

Brain proteome analysis of mice selectively bred for either high or low anxiety-related behavior revealed quantitative and qualitative protein expression differences. The enzyme glyoxalase-I was consistently expressed to a higher extent in low anxiety as compared with high anxiety mice in several brain areas. The same phenotype-dependent difference was also found in red blood cells with normal cross-mated animals showing intermediate expression profiles of glyoxalase-I. Another protein that showed a different mobility during two-dimensional gel electrophoresis was identified as enolase phosphatase. The presence of both protein markers in red or white blood cells, respectively, creates the opportunity to screen for the presence of both protein markers in red or white blood cells with normal cross-mated animals showing intermediate expression profiles of glyoxalase-I. Another protein that showed a different mobility during two-dimensional gel electrophoresis was identified as enolase phosphatase. The manifestation of anxiety in a number of psychiatric disorders such as generalized anxiety disorder, depression, panic attacks, phobias, obsessive-compulsive disorders, and post-traumatic stress disorder (3) highlights the importance of gaining a better understanding of associated reliable biomarkers in proper animal models. An animal model to study behavioral, neuroendocrine, and genetic concomitants of trait anxiety including psychopathology should represent a good approximation to score symptoms of anxiety disorders and possibly comorbid depression (4–6). To avoid interstrain comparisons, which are likely to reveal differences in more than just anxiety-related indices, we have been using intrastrain breeding approaches to focus on particular traits, including anxiety-related behavior (7), depression-like behavior (8), and avoidance behavior or receptor functions likely to be associated with differences in anxiety (9–12). The technique of selective bidirectional breeding enhances the representation of genetic material associated with a particular trait shifting the animals’ phenotype bidirectionally from the strain mean (13).

Genetic approaches currently available in the mouse make this model organism particularly powerful for the functional analysis of candidate genes and in defining gene products underlying trait anxiety and possibly depression (14). For this reason we have generated and validated hyperanxious (HAB-M)1 and hypoanxious (LAB-M) CD1 mouse lines as model of extremes in trait anxiety and have used comparative proteomics to identify anxiety-related protein markers (2). One of the identified markers that we have reported previously, glyoxalase-I (2), showed expression level differences between HAB-M and LAB-M animals and recently has attracted increasing attention for its role in psychopathogenic mechanisms (15). The other protein that exhibited a qualitative difference of unknown nature between the two mouse strains is enolase phosphatase.

EXPERIMENTAL PROCEDURES

Mice—The animal studies were conducted in accordance with the “Guide for the Care and Use of Laboratory Animals of the Government of Bavaria.” Mice were housed in groups of three to five in plastic cages (25 × 20 × 14 cm) with a 12-h light/dark schedule (lights on at 6 a.m.) at 23 °C and 60% humidity with food and tap water available ad libitum. Experiments were carried out between 8 a.m. and 12 p.m. As described previously (2) we used over 250 animals from more than 25 litters of the outbred Swiss CD1 strain (Charles River, Sulzfeld, Germany) as a starting point for selective and bidirectional breeding for anxiety-related behavior on the EPM at the age of 7 weeks. Males and females that spent either the least or most time on the open arms of the EPM were mated to establish the HAB-M and LAB-M mouse lines, respectively. The first generation (G1) was weaned and separated by sex at 3 weeks of age and tested on the EPM 4 weeks later.

1 The abbreviations used are: HAB-M, high anxiety-related behavior mouse; CM-M, cross-mated offspring; EP, enolase phosphatase; EPM, elevated plus-maze; GLX1, glyoxalase-I; LAB-M, low anxiety-related behavior mouse; NAB-M, normal anxiety behavior-related phenotype mouse; 2D, two-dimensional; TBST, Tris-buffered saline with Tween 20.
Again extremes were mated, and offspring were reared in the same manner for all following generations. Outbreeding across families but within closed lines was practiced to minimize random fixation of alleles other than those selected for and to also maximize the number of genes influencing anxiety-related behavior within each line. After nine generations of outbreeding, an inbreeding protocol was started based on strict sibling mating. We generated four to six independent families within the HAB-M and LAB-M lines, respectively. In the experiments, animals from all inbred HAB-M and LAB-M families from seven generations (G12–G18) were included to avoid both fortuitous correlations and fluctuations across time due to genetic drift (8).

CM-M offspring comprised reciprocal crosses between HAB-M and LAB-M mice. They were housed under the same conditions as HAB-M, LAB-M, and “normal” CD1 (NAB-M) animals, the latter being selected as controls independent of their performance on the EPM. All behavioral tests were conducted during weeks 7–13. Mice were initially tested on the EPM and, at least 2 days later, in one or more additional tests. The minimum recovery time between the tests was 1 day.

**Elevated Plus-Maze Test**—The EPM was made of black plastic and consisted of two open arms (30 × 5 cm; 300 lux) and two enclosed arms (30 × 5 × 15 cm; 10 lux). The arms extended from a central platform (5 × 5 cm; 90 lux). The EPM was located 120 cm above the floor. Male and female mice were placed into the EPM room 24 h prior to testing and stayed in their home cages until the experiment was carried out. At the beginning of the experiment, each mouse was placed on the central platform facing a closed arm. During the 5-min test, the percentage of time spent on the open arms, the number of entries into the closed and open arms, and the latency to the first open arm entry were scored using the computer software program “plus-maze” (E. Fricke, Munich, Germany). Mice were considered to have entered an open or closed arm when both front paws and front shoulders were on the arm, but also full entries (all four paws) were counted. After each test, the EPM was cleaned with water containing detergent and dried with tissue.

**Forced Swim Test**—Each mouse was placed in a glass cylinder (height, 23.5 cm; diameter, 11 cm) containing 15 cm of water at 22–23 °C for 6 min. At the end of each videotaped trial, immobility time (floating) was scored using the computer program Eventlog 1.0 (EMCO Software Ltd., Reykjavik, Iceland). A mouse was judged to be immobile when it stopped any movements except those that were necessary to keep its head above water.

**Protein Sample Preparation**—Three to five days after behavioral testing, HAB-M, NAB-M, CM-M, and LAB-M mice were sacrificed under isoflurane anesthesia, their trunk blood was collected (see “Western Blot”), and their brains were removed. The hypothalamus (containing the paraventricular nucleus and supraoptic nuclei), amygdala (containing the basolateral, central, and medial nuclei), and motor cortex were dissected according to the mouse brain atlas (16). The areas were weighed, immediately frozen in liquid nitrogen, and stored at −80 °C. Brain specimens were prepared for 2D PAGE with the PlusOne sample preparation kit (GE Healthcare) using IEF rehydration buffer (7 M urea, 2 M thiourea, 0.2% Biolytes 3–10, 2% CHAPS, 100 mM DTT supplemented with the protease inhibitor mixture Complete, PMSF, and pepstatin). Protein content of the samples was measured using the Bradford protein assay (Bio-Rad). The number of animals analyzed is given in the respective legends to the figures.

**Two-dimensional Polyacrylamide Gel Electrophoresis**—The brain area extracts (12 replicates for each HAB-M, LAB-M, and NAB-M group) with 300 μg of protein in IEF sample buffer were applied to a 17-cm, pH 4–7 Immobiline strip (Bio-Rad). After active rehydration at 50 V for 12 h paper wicks were used, and IEF was carried out at 20 °C in a Protean IEF cell (Bio-Rad) using the following conditions: 250 V for 15 min, 10,000 V for 3 h, and 10,000 V until 60,000 V h were reached. For reduction and alkylation of the proteins, the strips were equilibrated in 2% DTT for 30 min and then in 2.5% iodoacetamide for 30 min. For the second dimension SDS-polyacrylamide gel electrophoresis, strips were layered on top of a 5% stacking gel and 12% separating gel and were run in batches of 12 gels each at 50–200 V overnight.

Gels were stained with colloidal Coomassie Blue and scanned. The scanned images were analyzed and compared with each other with the help of PDQUEST software (Bio-Rad).

Spots were detected using the automated spot detection and matching function. After normalization according to total density spot intensities were quantitated and compared between the replicate groups (HAB-M, LAB-M, and NAB-M). Group differences were evaluated statistically applying Student’s t test with a significance level of 98% and the criterion of a minimum of 2-fold difference (increase or decrease) in spot intensity. The automated analysis was completed by manual matching and quantitation of spots of interest.

**Mass Spectrometry**—Gel spots that represented differences in protein expression levels based on the results from the image analysis were subjected to an in-gel tryptic digest. For this purpose, 50 μl of 20 mM ammonium bicarbonate/acetoneitrile (1:1) were used for gel cleanup and drying. After the gel pieces were dry, 30 μl of 20 mM ammonium bicarbonate containing 0.5 μg of trypsin (Promega, Madison, WI) were added and incubated at 37 °C overnight. Peptides were extracted twice with 25 μl of 5% formic acid by incubation at 37 °C for 30 min. The extracts were concentrated in a SpeedVac and redissolved in 12 μl of 0.1% formic acid. For mass spectrometry analysis, the tryptic digests were injected onto a C18 cartridge using a FAMOS autosampler (Dionex, Sunnyvale, CA). After washing the cartridge with 0.1% formic acid (solvent A) for 15 min, the peptides were eluted onto a PicoFrit column (New Objective, Woburn, MA) by applying a linear gradient of acetonitrile:water/formic acid (90:10:0.1) over 60 min. The PicoFrit eluate was directly infused into an LCQ DECA XP PLUS ion trap mass spectrometer (Thermo Electron, San Jose, CA). Each full scan was followed by a zoom scan and an MS/MS scan of the most intense ion. The resulting MS/MS data were used to search a non-redundant protein database, release date July 25, 2003 (National Center for Biotechnology Information (NCBI), Bethesda, MD), using TURBOSEQUENT (24). Mass spectrometry identifications were considered positive when a minimum of three peptides came up with the same protein hit.

**Western Blot**—Red and white blood cells were isolated from 0.8 ml of mouse (HAB-M, LAB-M, NAB-M, CM-M, C57BL/6J, and BALB/c) or patient blood that had been collected in EDTA tubes supplemented with Trasylol. The cell pellets were frozen at −80 °C until used. For red cell lysis, the pellets were thawed on ice, cold water containing 1 mM PMSF was added, and the suspension was mixed. Proteins from white blood cells were extracted with either IEF or SDS sample buffers. After the removal of cellular debris, the supernatant was stored in aliquots at −80 °C. For Western blot, 100 μg of total protein from each red or white cell lysate were run on either a 15% SDS minigel (Bio-Rad) or a 2D gel (Bio-Rad), and the proteins were transferred to an Immobilon PVDF membrane (Millipore, Bedford, MA) at 100 V for 1 h with cooling. The membrane was treated with 5% Carnation instant nonfat dry milk in TBST overnight and rinsed in TBST. The membrane was then incubated with either GLX1 antiserum (kindly provided by Dr. Kenneth Tew, Fox Chase Cancer Center, Philadelphia, PA) or EP antibody (MorphoSys, Martinsried, Germany) at a 1:2,000 dilution in TBST for 2 h at room temperature and washed with water and TBST for 15 min. Incubations with either protein A- horseshadish peroxidase (for GLX1) or an anti-c-MYC-peroxidase antibody (for EP) (GE Healthcare and Roche Diagnostics) were carried out for 40 min at room temperature after which the membrane was washed with water and TBST. Finally the membrane was incubated...
with ECL mixture (GE Healthcare) for 1 min and exposed to ECL film (GE Healthcare). The membrane was scanned, and the band signal strength (optical density) was assessed with the help of the QuantityOne software (Bio-Rad).

**RESULTS**

In our efforts to identify biomarkers for trait anxiety, a mouse model was established by using a bidirectional in-breeding protocol (2). Anxiety-like behavior on the elevated plus-maze was the key selection criterion (Fig. 1A). The increasing divergence between HAB-M and LAB-M animals is depicted in Fig. 1B with the former showing significantly heightened anxiety-related behavior compared with their LAB-M counterparts (G4–G21: \( p < 0.001 \)). The time outbred CD1 mice spent on the open arms of the EPM ranged from 0 to 54.6% for males and from 0 to 65.1% for females. Independent of gender, both selected lines differed significantly from unselected NAB-M controls as shown in Fig. 1B.

Male and female LAB-M mice also displayed significantly less immobility time than HAB-M, NAB-M, and CM-M mice in a depression-related behavioral assay, the forced swim test (males: \( F_{3.138} = 41.3 \), females: \( F_{3.115} = 61.9, p < 0.001 \); Fig. 2). Individual pairs of data (percentage of time in the EPM versus time spent immobile in the forced swim test) revealed a significant correlation (2). Both behavioral assays, the EPM and the forced swim test, showed a very consistent difference in the phenotype of the two mouse strains, which was a prerequisite for subsequent proteomics analyses.

For biomarker identification, protein extracts from individual
brain areas from HAB-M and LAB-M mice were subjected to 2D PAGE (Fig. 3). The first dimension IEF was run with a pH gradient of 4–7, and the second dimension was run in a 12% SDS gel. After staining the gels with colloidal Coomassie Blue, the scanned images were compared by computer analysis. Each gel contained ~800 detectable protein spots. As we have reported previously (2) one of the protein spots differed significantly in its intensity between all the HAB-M and LAB-M animals in all three brain areas analyzed (hypothalamus, amygdala, and motor cortex). To obtain a good estimate of the relative expression levels, we used Western blot analysis (2). Based on the signal intensity of the GLX1 protein band, the level of expression of this protein was ~10-fold higher in LAB-M compared with HAB-M animals (Fig. 4, A and B). Another protein showed a different migration pattern in the gels indicating a qualitative variance that is either due to an altered post-translational modification or an allelic difference. In HAB-M animal tissue extracts, the EP protein exhibited a slower mobility in the second dimension than for LAB-M and NAB-M mice. No significant difference in the spot intensities was observed for EP between the three mouse lines (LAB-M: 0.123, HAB-M: 0.180, NAB-M: 0.133; Fig. 3). The exact nature

![Representative 2D PAGE of amygdala protein extracts from male HAB-M (A), LAB-M (B), and NAB-M (C) animals. The differences in expression of GLX1 and EP protein spots are pointed out with arrows. The basic end of the gel is on the left side, and the acidic end is on the right side of the gels. Results represent data from G12; a total of 12 animals were analyzed for each group.](Image)

![Expression analysis by Western blot. A, red blood cell protein extracts from male HAB-M, NAB-M, CM-M, and LAB-M animals representing equal amounts of total protein (100 μg) were run on a 12% SDS gel and transferred to a PVDF membrane. Subsequently the membrane was probed with an anti-GLX1-specific antibody and developed. The immunoreactive GLX1 protein bands are shown (adapted from Krömer et al. (2) with permission). B, the signal volumes of the bands obtained from GLX1 Western blot analysis in A were quantified with a densitometer. C, EP Western blot of amygdala protein extracts from male HAB-M and LAB-M mice. Extracts were subjected to 2D PAGE and transferred to a PVDF membrane. The membrane was probed with an anti-EP-specific antibody and developed. The additional EP isoform spot in HAB-M mice that is missing in LAB-M mice is indicated with an arrow.](Image)
of the observed difference in migration will be determined in future experiments. Both marker proteins were identified by cutting out the stained gel spots from HAB-M, LAB-M, and NAB-M tissue extract gels and subjecting the proteins to in-gel digestion with trypsin followed by tandem mass spectrometry. Mass spectrometry data were used to search the non-redundant protein database and resulted in the identification of GLX1 (2) and EP, respectively. The following peptide sequences were identified for the two proteins: GLX1: SLDFYTR, VLGLTLQQK, RFEELQGK, and DFLLQTLMLR; EP: QLQGHMWK, AAFTAGR, VDSESyr, and PGNAoLTDEK.

Fig. 5. GLX1 (A) and EP (B) Western blot analyses of human red (A) and white (B) blood cell protein extracts from four individuals (1–4 and a–d). Immunoreactive bands that represent GLX1 (A) and EP (B) are indicated with arrows.

This represents an 18.48 and 12.84% amino acid coverage for GLX1 and EP, respectively. Because 2D PAGE is a rather laborious method for studying protein expression in tissues, we next wished to set up a high throughput method for the quantitative analysis of GLX1 and EP. Because GLX1 is expressed ubiquitously in many cells and tissues (17), we were able to use red blood cells as a source for these experiments. The protein sequence of GLX1 is highly conserved among higher vertebrates; this allowed us to use a polyclonal antiserum that was generated against human GLX1 for the detection of the mouse enzyme by Western blot analysis (Fig. 4A) (2). In the case of EP we obtained an antibody from a commercial source. Because EP is not expressed in red blood cells, we used HAB-M and LAB-M mouse brain tissue to validate the expression difference that we had observed in 2D PAGE (Fig. 4C).

In the case of GLX1 the Western blot enabled us to screen a great number of mouse red blood cell specimens (2). To extend our studies, we also included samples from normal NAB-M and cross-mated CM-M controls, which displayed intermediate scores in the EPM test (Fig. 4A). Independent of their gender, both groups, NAB-M and CM-M animals, also showed intermediate levels of GLX1 expression as compared with LAB-M and HAB-M mice, the former again expressing significantly more GLX1 thus confirming the brain tissue data (Fig. 4, A and B) (2).

Enolase phosphatase Western blot analysis of HAB-M and LAB-M mouse brain tissue revealed a complex expression pattern when 2D PAGE was used for protein separation (Fig. 4C). Despite its complexity an additional spot that migrates slower in the second dimension SDS gel was detected in HAB-M brain tissue extracts and was absent in LAB-M extracts. This isofrom pattern is in agreement with the one observed in the original 2D PAGE analysis of brain tissue from the two mouse strains (Fig. 3). The nature of the different isofrom in HAB-M animals is currently under investigation.

In an extension of our studies we have begun to screen human specimens with the long term goal to correlate the expression of biomarkers that were identified in mouse models with disease phenotypes in humans. Working to our advantage both GLX1 and EP are expressed in blood cells, which are isolated on a routine basis in clinical laboratories. Whereas GLX1 is found in red blood cells, EP expression can be analyzed in white blood cells as a source. Preliminary screens with a limited number of patient specimens showed that both proteins can be detected by Western blot analysis from these cells (Fig. 5). In the case of EP the two immunoreactive bands comigrated with the two spots that were originally detected during 2D PAGE of the HAB-M and LAB-M amygdala extracts. Due to the small amount of patient blood specimens, we were unable to produce a Coomassie-stained 2D gel of the white blood cell extracts.

DISCUSSION

Selective and bidirectional breeding of CD1 mice for either high or low anxiety-related behavior resulted in two inbred lines that, independent of their gender, differ extremely in a variety of behavioral paradigms (2). The EPM, based on spontaneous, unconditioned behavior, was selected as an initial key evaluation tool. LAB-M mice were not only less anxious compared with HAB-M mice in the EPM test but also showed lower immobility scores in the forced swim test, indicative of a reduced depression-like behavior (2). Using 2D PAGE of several brain tissue sections (hypothalamus, amygdala, and cortex) and subsequently Western blot analysis, GLX1 (2) and EP were identified as protein markers, which are consistently expressed at a different level or with an altered pattern, respectively. Other spot differences were not observed in a consistent manner. In our experience the method of 2D PAGE followed by Coomassie Blue staining is only capable of detecting significant differences of abundant proteins. For a more sensitive analysis of the trait anxiety mouse model proteins, we are planning to use a stable isotope-based proteomics platform in the future.

The significant correlation between anxiety- and depression-related behaviors in HAB-M and LAB-M mice resembles the clinical situation of a high comorbidity between anxiety disorders and depression (18). The correlation in mice, however, is primarily due to the extremely low depression-like behavior of LAB-M, whereas HAB-M mice did not significantly differ from NAB-M and CM-M controls, respectively. Thus,
although HAB-M and LAB-M mice selectively bred for a specific anxiety-related phenotype using the EPM show similar differences across a variety of anxiety- and depression-related behavioral paradigms, HAB-M animals show little difference from NAB-M or CM-M controls in the depression-related forced swim test (2). These findings suggest that test-dependent facets of behavior are involved with at least partially different genetic mechanisms controlling the various anxiety- and depression-related indices.

A major goal in the area of mental disorders is the identification of biomarkers that can categorize subsets of subjects in a more reliable and consistent manner. Our approach demonstrates that proteomics analyses are suited to identify protein markers that are differentially expressed in CD1 mice with different genetic predispositions to anxiety-related behavior. GLX1 represents an enzyme in the cytosolic fraction of cells and tissues of many organisms (17, 19). Although its function is not entirely clear, it has been shown that the enzyme plays a major role in the detoxification of methylglyoxal, which represents a potent cytotoxic metabolite. GLX1 catalyzes the transformation of methylglyoxal and glutathione to S-lactoylglutathione, which is converted to d-lactic acid by glyoxalase-II (19). Due to its ubiquitous expression, the methylglyoxal pathway is believed to be of fundamental importance for cellular metabolism. GLX1 was identified as the only up-regulated gene in mutant mice sharing common pathogenic mechanisms with Alzheimer patients (15). A possible connection between GLX1 and depression has been found in a linkage study of families with depressive disease. Subgroups of families with unipolar affective disease showed evidence for a linkage or association with the GLX1 locus (20). In a recent study using different inbred strains of mice Hovatta et al. (21) found that GLX1 and glutathione reductase 1 play a causal role in anxiety. It remains to be seen whether GLX1 represents a risk marker or a risk factor for the anxiety-related phenotype in mice (22).

In our newly developed mouse model of extremes in trait anxiety, GLX1 in brain areas and blood cells has been shown to represent a robust, reliable biomarker for the non-anxious versus anxious phenotype (2). Although expressed at an intermediate level in unselected NAB-M as well as CM-M controls, it showed a bidirectional shift from the strain mean toward reduced (HAB-M) and increased (LAB-M) expression levels, thus mimicking the anxiety phenotype (2).

Very little has been reported on the function of the other marker protein that was identified in our analysis. As its name indicates, EP is an enzyme that has both enolase and phosphatase activities and is involved in metabolic pathways of energy production and conversion (23). No association with any disease has been reported to date. The nature of the different isoforms that were found in HAB-M and LAB-M mice is currently under investigation.

The expression of both marker proteins, GLX1 and EP, can be assessed in human specimens by isolating red and white blood cells, respectively. Future studies with a great number of case and control specimens will determine whether the two proteins can be used in the diagnosis of anxiety disorders in patients.

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† To whom correspondence should be addressed: Max Planck Inst. of Psychiatry, Krapelininstr. 2, D-80804 Munich, Germany. Tel.: 49-89-30622317; Fax: 49-89-30622200; E-mail: turck@mipsykl.mpdl.mpg.de.

REFERENCES

1. LaBaer, J. (2005) So, you want to look for biomarkers. J. Proteome Res. 4, 1053–1059
2. Krömer, S. A., Kessler, M. S., Millyart, D., Birg, I. N., Bunck, M., Czibere, L., Panhuysen, M., Pütz, B., Deussing, J. M., Holsboer, F., Landgraf, R., and Turck, C. W. (2005) Identification of glyoxalase-I as a protein marker in a mouse model of extremes in trait anxiety. J. Neurosci. 25, 4375–4384
3. Gross, C., and Hen, R. (2004) The developmental origins of anxiety. Nat. Rev. Neuroscience 5, 545–552
4. Finn, D. A., Rutledge-Gorman, M. T., and Crabbé, J. C. (2003) Genetic animal models of anxiety. Neurogenetics 4, 109–135
5. Oryan, J. F., and Mombereau, C. (2004) In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. Mol. Psychiatry 9, 326–357
6. Gordon, J. A., and Hen, R. (2004) Genetic approaches to the study of anxiety. Annu. Rev. Neurosci. 27, 193–222
7. Landgraf, R., and Wigger, A. (2003) Born to be anxious: neuroendocrine and genetic correlates of trait anxiety in HAB rats. Stress 6, 111–119
8. El Yacoubi, M., Bouali, S., Popa, D., Naudon, L., Leroux-Nicollet, I., Hamon, M., Costentin, J., Adrien, J., and Vaugeois, J.-M. (2003) Behavioral, biochemical, neurochemical, and electrophysiological characterization of a genetic mouse model of depression. Proc. Natl. Acad. Sci. U. S. A. 100, 6227–6232
9. Ohita, R., Shirota, M., Adachi, T., Tohei, A., and Taya, K. (1999) Plasma ACTH levels during early, two-way avoidance acquisition in high- and low-avoidance rats (Hatano strains). Behav. Genet. 29, 137–144
10. Brush, F. R. (2003) The Syracuse strains, selectively bred for differences in active avoidance learning, may be models of genetic differences in trait and state anxiety. Stress 6, 77–85
11. Overstreet, D. H., Commissaris, R. C., De La Garza, R., II, File, S. E., Knapp, D. J., and Seiden, L. S. (2003) Involvement of 5-HT1A receptors in animal tests of anxiety and depression: evidence from genetic models. Stress 6, 101–110
12. Steimer, T., and Driscoll, P. (2003) Divergent stress responses and coping styles in psychogenetically selected Roman high-(RHA) and low-(RLA) avoidance rats: behavioural, neuroendocrine and developmental aspects. Stress 6, 87–100
13. Falconer, D. S., and Mackay, T. F. C. (1996) Introduction to Quantitative Genetics, 2nd Ed., Addison Wesley Longman, Harlow, Essex, UK
14. Tarantino, I. M., and Bucan, M. (2000) Dissection of behavior and psychiatric disorders using the mouse as a model. Hum. Mol. Genet. 9, 953–965
15. Chen, F., Wollmer, M. A. Hoermdd, F., Munch, G., Kuhla, B., Rogae, E. I., Tsolaki, M., Papassotropoulos, A., and Gotz, J. (2004) Role for glyoxalase-I in Alzheimer’s disease. Proc. Natl. Acad. Sci. U. S. A. 101, 7687–7692
16. Paxinos, G., and Franklin, K. (2001) The Mouse Brain in Stereotaxic Coordinates, Academic Press, San Diego, CA
17. Hayes, J. D., Milner, S. W., and Walker, S. W. (1989) Expression of glyoxalase, glutathione peroxidase and glutathione S-transferase isoenzymes in different bovine tissues. Biochim. Biophys. Acta 994, 21–29
18. Levine, J., Cole, D. P., Chengappa, K. N., and Gershon, S. (2001) Anxiety...
disorders and major depression, together or apart. *Depress. Anxiety* 14, 94–104
19. Thornalley, P. J. (1993) The glyoxalase system in health and disease. *Mol. Aspects Med.* 14, 287–371
20. Tanna, V. L., Wilson, A. F., Winokur, G., and Elston, R. C. (1989) Linkage analysis of pure depressive disease. *J. Psychiatr. Res.* 23, 99–107
21. Hovatta, I., Tennant, R. S., Helton, R, Marr, R. A., Singer, O., Redwine, J. M., Ellison, J. A., Schadt, E. E., Verma, I. M., Lockhart, D. J., and Barlow, C. (2005) Glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice. *Nature* 438, 662–666
22. Thornalley, P. J. (2006) Unease on the role of glyoxalase 1 in high-anxiety-related behaviour. *Trends Mol. Med.* 12, 195–199
23. Wang, H., Pamg, H., Bartlam, M., and Rao, Z. (2005) Crystal structure of human E1 enzyme and ist complex with a substrate analog reveals the mechanism of ist phosphatase/enolase activity. *J. Mol. Biol.* 348, 917–926
24. Yates, J., III, and Eng, J. (1994) TURBOQUEST, University of Washington, Seattle, WA