Send Orders for Reprints to reprints@benthamscience.net

Current Neuropharmacology, **2014**, *12*, **193-204**

Oxidative Imbalance and Anxiety Disorders

R. Krolow*, D. M. Arcego, C. Noschang, S. N. Weis and C. Dalmaz*

Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, UFRGS, Porto Alegre, Rio Grande do Sul, Brazil

Abstract: The oxidative imbalance appears to have an important role in anxiety development. Studies in both humans and animals have shown a strong correlation between anxiety and oxidative stress. In humans, for example, the increased malondialdehyde levels and discrepancies in antioxidant enzymes in erythrocytes have been observed. In animals, several studies also show that anxiety-like behavior is related to the oxidative imbalance. Moreover, anxiety-like behavior can be caused by pharmacological-induced oxidative stress. Studies using knockout or overexpression of antioxidant enzymes have shown a relationship between anxiety-like behavior and oxidative stress. Related factors of oxidative stress that could influence anxious behavior are revised, including impaired function of different mitochondrial proteins, inflammatory cytokines, and neurotrophic factors. It has been suggested that a therapy specifically focus in reducing reactive species production may have a beneficial effect in reducing anxiety. However, the neurobiological pathways underlying the effect of oxidative stress on anxiety symptoms are not fully comprehended. The challenge now is to identify the oxidative stress mechanisms likely to be involved in the induction of anxiety symptoms. Understanding these pathways could help to clarify the neurobiology of the anxiety disorder and provide tools for new discovery in therapies and preventive strategies.

Keywords: Antioxidants, anxiety disorders, anxiolytics drugs, genetics, inflammation, mitochondrial, neurotrophic factor, reactive species.

INTRODUCTION

Anxiety is defined as a state of uneasiness and apprehension. It is a general term that describes a large range of related and commonly experienced subjective mental states which are normally evoked by a wide range of external and internal stressors. Anxiety is a normal emotional response but when it is inappropriate (at in inappropriate time or to an inappropriate degree, and is disruptive to the individual), it then constitutes an anxiety disorder [1, 2]. This condition is implicated in a number of psychiatric disorders, such obsessive compulsive disorder (OCD), panic disorder (PD), social phobias (SP), generalized anxiety disorder (GAD) and post-traumatic stress disorder (PTSD) [1].

Genetic and environmental factors may be involved in the development of anxiety. Hovatta *et al.* [3], have shown that local inhibition of glyoxalase 1 expression by RNA interference decreased the anxiety-like behavior. In addition, chronic restraint stress and consumption of palatable diets also induce anxiety-like behaviors in adult rats [4-6].

The role of oxidative stress in psychiatric and neurological disorders, including anxiety, has been the focus of many investigations. The brain is especially sensitive to oxidative damage and has relatively modest antioxidant defenses [7]. The brain is rich in lipid substrates for oxidation, and iron and copper that catalyze free radical reactions. It also requires large amounts of oxygen to function normally, and therefore produces a comparatively large amount of free radical by-products. In addition, one of the major sources of free radicals in the brain is the metabolism of catecholamines, neurotransmitters released in anxiety states. This metabolism appears to be associated with the increased oxidative damage [8, 9].

Numerous physiological and pathological processes, such as, emotional or psychological stress, psychiatric disorders, ageing, excessive caloric intake, infections, inflammatory disorders, and pharmacological treatments, increase the bodily concentration of oxidizing substances, known as free radicals or reactive species [10]. Where do these species come from? The production of cellular energy (in the form of ATP) *via* the process of mitochondrial oxidative phosphorylation is absolutely essential for normal cellular function. However, it is well known that mitochondrial oxidative phosphorylation system generates reactive oxygen species (ROS) and reactive nitrogen species (RNS) [11]. These are chemical species highly reactive owing to the presence of free unpaired electrons. The production of these reactive species has both beneficial and harmful roles to the cells. Lower concentrations of these species are involved in the regulation of a number of physiological processes such as cellular response to injury or infection and cellular signaling [12]. However, overproduction results in oxidative injury which can be an important mediator of damage to cell structures like lipids, membranes, protein and DNA [12]. This oxidative imbalance between cellular levels of reactive species and cellular antioxidant defenses may also result from altered defense mechanisms, such as depletion of

*Address correspondence to these authors at the Departamento de Bioquímica, ICBS, UFRGS, Ramiro Barcelos, 2600 (Anexo) Lab. 37. 90035-003 Porto Alegre, RS, Brasil; Tel: 55 51- 3316-5570; Fax: 55 51- 3316-5535;
E-mails: Krolowrachel@yahoo.com.br, carladalmaz@yahoo.com.br

1875-6190/14 $58.00+.00

enzymatic (e.g., superoxide dismutase [SOD], catalase [CAT], and glutathione peroxidase [GPX]), and non-enzymatic (e.g., glutathione [GSH] vitamins A, C, and E, and selenium) antioxidants, namely oxidative stress. This process may be involved in the pathogenesis of several brain diseases, including anxiety [13-18].

Here, we outline some of the findings in oxidative stress and anxiety, considering possible connections between these conditions. Factors related to oxidative stress and that could influence anxiety are revised.

ANXIETY AND OXIDATIVE STRESS IN HUMAN STUDIES

A correlation between reactive species production and these diseases has been postulated. An important marker of oxidative damage is malondialdehyde (MDA) levels, which were found increased in patients with disorders associated to anxiety. MDA is a product of lipid peroxidation followed the reaction between lipids and free radicals. The measurement of MDA is considered an index of oxidative change of lipids and thereby of oxidative stress [19]. In addition, this product of lipid peroxidation may be present in the blood and urine [20]. Bulut and colleagues [21] suggested that the increased MDA could be related to the pathophysiology of some psychiatric diseases. Chakraborty and colleagues [22] showed that an increase in OCD severity in patients might be associated with increased lipid peroxidation markers in the serum. The authors suggested that this increment could be induced by neuronal cell damage in certain areas of the brain. Another study also showed that MDA levels were significantly higher in patients with OCD [23]. Additionally, a study showed that caregiving to a family member with advanced cancer, which is considered a very stressful activity, also induced the increase in MDA levels in urine during anxiety and depression [24].

It has been shown that anxious women present an increased respiratory burst, with consequent generation and release of superoxide anion [25]. Decomposition of superoxide into hydrogen peroxide (H_2O_2) is catalyzed by SOD, and several studies have detected increased activity of this enzyme in response to increased ROS production in psychiatric patients [26-29].

Some discrepancies regarding antioxidant enzymes activities, such as GPx and CAT, have been found in patients. These enzymes are important for the oxidative balance, since they help in the degradation of H_2O_2 into water. Reports showed lower activity of GPx in erythrocytes of patients with anxiety disorders in comparison with healthy controls [30, 31]. On the other hand, other studies showed higher GPx activity in erythrocytes of patients with OCD or social phobia (SP) [32, 33]. Also, some studies found no change in the CAT activity [28, 34, 35], while other studies showed an increased activity of this enzyme in erythrocytes of patients with social phobia [33].

It is important to mention that those conflicted finding only correlations and that those measurements were mostly done in serum, plasma and blood cells, and a few studies try to correlate these findings with oxidative stress in brain. Nevertheless, animal models have been adding more information regarding anxiety and oxidative stress.

ANXIETY AND OXIDATIVE STRESS IN ANIMAL MODELS

Many studies, using different protocols, have suggested a correlation between anxiety and oxidative stress in animals. On the other hand, there are only a few studies evaluating the oxidative stress consequences on anxiety-like behavior in rats. Here we will highlight some of these two types of study.

Oxidative stress and anxiety-like behavior in animals have been described in a variety of paradigms. For example, vitamin A supplementation in rats appears to increase hippocampal lipid peroxidation, protein carbonylation and oxidation of protein thiol content (all these result from oxidative damage). In addition, increased SOD, decreased CAT activities, and anxiogenic behavior were also observed [36]. Perinatal ethanol exposure induced depressive and anxiety-like behaviors in adult rats, associated with changes in oxidative stress parameters such as, increased levels of lipid peroxidation and protein oxidation, and reduction in glutathione levels in the hippocampus and cerebellum [37]. Acute administration of adriamycin, an effective anti-cancer chemotherapy drug used in humans, increased anxiety-like behavior was showed, and impaired the locomotor and exploratory activities in male rats, when compared to controls. Additionally, drug-treated animals revealed an increased glutathione-S-transferase activity and MDA levels, while brain glutathione concentration decreased, when compared to controls [38].

It has been shown that naive Swiss albino male mice present a large heterogeneity in their anxiety levels [39]. Therefore, these mice with different levels of anxiety have been selected to evaluate the brain and peripheral oxidative parameters. The anxiety levels in those mice were associated with the oxidative status in both neuronal and glial cells in the cerebellum and hippocampus, in cortical neurons and in peripheral leucocytes (monocytes, granulocytes and lymphocytes), revealing increased levels of reactive oxygen species in the brain and in the peripheral system of anxious mice [40]. On the other hand, mice overexpressing mitochondrial CAT exhibited reduced anxiety in the elevated zero maze [41]. CAT activity was increased in the brain of these animals and no difference in glutathione, protein carbonyl, lipid peroxidation and 8-hydroxyguanine levels were observed between groups in [41].

Studies have shown a relation between oxidative stress and anxiety in animals exposed special diet or stress. Rats fed with a methionine enriched-diet presented increased levels of anxiety evaluated in an elevated plus maze test and increased superoxide dismutase (SOD) activity in the cerebral cortex when compared to controls [42]. Another study suggested that the highly palatable diet (enriched with sucrose) intake led to an obese phenotype, increased protein oxidation in frontal cortex and appeared to induce anxiety-like behavior in rats [5]. It has been also shown that psychological stress (using a communication box paradigm) induced oxidative damage in masseter muscles in rats that

was associated with behavior resembling anxiety. Stressed rats exhibited a decreased percentage of time spent in open arms in the elevated plus-maze test, decreased time spent in the center zone in the open field. These animals also presented, decrease in SOD, glutathione peroxidase and CAT activities and an increased in MDA content in the masseter muscle compared to controls [43]. A study using a different stress model, the chronic restraint stress, showed that male stressed rats had an increased anxiety-like behavior, and increased DNA fragmentation in the hippocampus [6]. The same effects were observed with chronic intake of caffeine. In both studies the authors suggested that the increase in DNA fragmentation was associated with the oxidative stress [6, 44].

One aspect that seems contrary to the idea that increased anxiety is related to oxidative stress, is the studies in male rats with 5,7-dihydroxytryptamine -induced lesion in the hypothalamic paraventricular nucleus (PVN) reporting a decreased anxiety-like behavior suggested by an increased time spent in the open arms in the elevated plus maze test, when compared to sham-operated rats. Additionally, although no significant changes in SOD or glutathione peroxidase activities in the temporal lobe, an increased level of MDA was observed in these PVN-lesioned animals [45]. As PVN is essential to the stress response [46], which is related to anxiety [47]; this type of lesion could be masking the effect of oxidative stress on anxiety.

To test the direct involvement of oxidative stress in anxiety-like behavior, rats received intraperitoneal injections at non-toxic dose of L-buthionine-(S,R)-sulfoximine (BSO), an agent that increases oxidative stress markers [48]. As a result, increased anxiety-like behavior was observed in rats receiving BSO compared to vehicle-treated animals. Additionally, two weeks of treatment with antioxidant prior to BSO injection was able to attenuate the BSO-induced anxiety-like behavior, suggesting the participation of oxidative stress in this phenomenon [48]. Rats exposed to a different protocol of oxidative stress induction (xanthine 0.1% in the drinking water and intraperitoneal injections of xanthine oxidase), had increased oxidative stress markers, 8-isoprostane (in serum and urine) and MDA (in hippocampus and amygdala), when compared to controls. In addition they also presented increased anxiety like-behavior observed in the open-field and light-dark exploration behavior tests [49]. The possible mechanism of oxidative stress to induce anxiety, could be an alteration in cyclic nucleotide signaling. It has been shown that oxidative stress leads to anxiogenic behavior in mice, which is reversed by phosphodiesterase-2 inhibition (an enzyme that regulates cGMP and cAMP signaling), probably through an increase in cGMP-protein kinase G signaling [50].

ANXIETY, OXIDATIVE STRESS AND MITOCHONDRIA

During stressful conditions, overstimulation of glutamatergic transmission causes a sustained intracellular calcium influx leading to excessive mitochondrial calcium sequestration [51], mitochondrial swelling, depolarization, oxidative phosphorylation uncoupling and oxidative damage [52, 53]. Several investigations have proposed that an impaired mitochondrial function is related to psychiatric and neurodegenerative disorders [54-58]. Mitochondria are organelles crucial not only for energy production, but also for the metabolism of amino acids, lipids and steroids. In addition, the mitochondrion controls free radicals levels (as discussed above), and regulates neurotransmitters, intracellular calcium concentration and apoptosis cell death processes. It is also involved in neuronal development, synaptogenesis, synaptic development and plasticity [59-61].

In a experimental study using mice, high anxiety-related behavior was associated to decreased glycolysis enzymes levels, increased expression of electron transport chain components and higher expression of proteins participating in transport into and within mitochondria in cingulated cortex. These features could lead to enhanced ROS production and oxidative stress, which result in oxidative damage, lipid peroxidation, and cell death [62].

As mentioned above (see oxidative stress in animal models), exposure to stressors is an important factor that induces anxiety-like behavior. It has been shown that corticosterone, the glucocorticoid hormone secreted during stress in rodents, regulates mitochondrial function, with neuroprotective [63, 64], or toxic effects [64, 65]. It was also noted that the glucocorticoid receptor (GR) is able to form a complex with the antiapoptotic protein Bcl-2, facilitating its translocation into mitochondria, in a biphasic relationship with concentrations of corticosterone [64]. In rodents chronically treated with corticosterone, GR levels in mitochondria were significantly reduced, with high doses of corticosterone also reducing mitochondrial Bcl-2 levels, thereby decreasing mitochondrial function [64].

The mitochondria are also important for the biosynthesis of neurosteroids [66]. The rate-limiting step in neurosteroids synthesis is the translocation of the substrate cholesterol from the outer to the inner mitochondrial membrane, mediated by Translocator Protein (TSPO), formerly called peripheral-type or mitochondrial benzodiazepine receptor [67-70]. TSPO is implicated in many mitochondrial functions, such as cholesterol transport, steroid hormone synthesis, mitochondrial respiration, mitochondrial permeability transition pore (mPTP) opening, apoptosis and cell proliferation [71-74]. TSPO has also been suggested to be involved in various anxiety-related diseases as social phobia and panic disorder [75-77]. Some disorders, including panic disorder, bipolar disorder and schizophrenia – all associated with anxiety symptoms – exhibit downregulation of platelet and/or lymphocyte TSPO [78]. In the presence of TSPO ligands, a neuroprotective effect was observed, through modulation of inflammatory and gliosis responses, reducing panic and anxiety symptoms [79-86]. In addition, some TSPO ligands exert beneficial effects, such as inhibition of cytochrome *c* release, and blockade of injury-induced membrane permeability transition pore opening [87, 88]. TSPO ligands efficiently increase neurosteroidogenesis in rat brain [81, 89-92], and because neurosteroids are GABA agonists, its TSPO-dependent upregulation may modulate GABAergic neurotransmission, alleviating anxious symptoms.

Another mitochondrial protein that has been related to anxiety and to oxidative stress is uncoupling protein-2

(UCP2) [93-95]. This protein is a member of the larger family of mitochondrial anion carrier proteins; it is expressed in neurons of stress-related regions of the hypothalamus-pituitary-adrenal (HPA) axis, and in the limbic system [93]. UCP2-deficient mice show significantly more anxiety-like behavior in the elevated plus maze [93]. Additionally, UCP2 deficiency in mouse brain is linked to alterations in dopamine turnover, reduced mitochondrial number in dopamine neurons and high ROS production (the primary function of UCP2 is to limit ROS production) [94, 95].

Therefore, an impaired mitochondrial function may be related to anxious behavior through the function of different mitochondrial proteins, besides its known role in the generation of ROS [62].

ANXIETY, OXIDATIVE STRESS AND INFLAMMATION

Some disabling psychiatric disorders related to anxiety, such as PTSD, PD, OCD and GAD, appear to have a strong immunological component. Patients with PTSD showed high levels of circulating C-reactive protein, interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), interleukin 1b (IL-1b) and interleukin 8 (IL-8), probably due to dysregulation of the immune system [96-100]. Regarding OCD data, ambiguous outcomes were found. Results from patients with OCD showed decreased TNF-α and natural killer activity and also a trend to IL-6 reduction [101, 102]. In contrast, raised levels of TNF-α and IL-6 were demonstrated in OCD patients, probably due to a high co-morbidity of depressive disorder [103].

Oxidative stress is one possible mechanism that may be related to inflammation and development of anxious behavior. Studies showed that inflammatory cytokines may be increased after oxidative stress [104-107], and may induce anxiety [108]. In addition, quercetin, which has anti-inflammatory properties [109], also has a role as an antioxidant agent [109, 110] and reverses stress-induced anxiety-like effects [110]. The nuclear factor κB (NFκB), an important mediator to inflammatory response, is activated upon oxidative stress and appears to lead to inflammation and neuronal damage [111, 112]. Collaborating with these findings, Salim and colleagues [113] showed that sub-chronic oxidative stress promotes NFκB-dependent inflammation and contribute to induce anxiety-like behavior in rats.

In general, results regarding the role of inflammation in anxiety disorders suggest a relation between these two conditions. Mice expressing high cytokines levels present enhancement of anxiety behavior [114-116], and deletion of interferon-gamma (IFN-γ) also increased anxiety [117]. Overexpression of IL-6 or TNF-α leads to an anxiogenic phenotype [118, 119]. Additionally, we must consider that a deleterious outcome on immune system is related to human chronic anxious state [120-123].

Inflammation may have a straight contribution on the neurobiology and pathogenesis of anxiety, given that cytokines can change HPA axis function, stimulating the expression and release of corticotropin-releasing hormone, adrenocorticotropic hormone and cortisol [124, 125]. In addition, evidences on clinical and experimental studies indicate that stress can activate an inflammatory cascade, demonstrated by rise in circulating concentrations of pro-inflammatory cytokines [126]. Authors have been demonstrated that mouse model of induced sepsis exhibited late anxiety-like behavior accompanied by high levels of brain TNF-α, interferon-c (IFN-c), IL-1b and IL-6 and elevated serum levels of TNF-α [127]. An anxiety-like behavior in mice following persistent inflammatory pain was found with concomitant TNF-α increased in basolateral amygdala. Local infusion of the TNF-α neutralizing antibody, infliximab, reversed the anxiety-like behavior [128], suggesting that this type of behavior could be induced by increased levels of TNF-α.

ANXIOLYTIC DRUGS AND OXIDATIVE STATUS CHANGES

Anxiolytic drugs have been reported to present distinct effects on the oxidative status. The effect of a single dose of diazepam (given to the animals before the initiation of stress) was tested on different markers of oxidative damage in the striatum of rats in an acute model of immobilization stress [129]. It was showed that the stress produced enhanced lipid peroxidation levels, decreased superoxide dismutase activity and reduced mitochondrial function, while pre-treatment of stressed rats with diazepam decreased lipid peroxidation levels [129]. Diazepam itself produced decreased mitochondrial function, suggesting a direct effect of this drug on the mitochondrial reductive capacity [129]. On the other hand, other study has shown that a single dose of diazepam caused free radical mediated changes, increasing or decreasing oxidative damage depending on the cellular fraction analyzed. This modulatory response of antioxidant defenses appeared to be region specific [130]. Chronic administration of diazepam increased levels of lipid peroxides and decreased activity of SOD. Also, a decreased glutathione levels, were observed in rat liver, when compared to controls, suggesting that chronic diazepam may be an oxidative stress inducer [131].

Researchers have been evaluating the effect of other benzodiazepine, the alprazolam, on the levels of intracellular ROS in the peripheral blood leukocytes of stressed mice. Alprazolam was given 30 min before exposing the animals to acute stress (immobilization for 6h). Stress increased the generation of ROS in peripheral defense cells, while treatment with alprazolam partially reversed it [132]. With a similar protocol, acute immobilization stress led to increased MDA and nitrite levels, and depleted glutathione levels and CAT activity in the brain, while pretreatment with alprazolam decreased MDA and nitrite levels and restored glutathione levels and CAT activity, suggesting that alprazolam has a neuroprotective effect [133]. The protective effects of benzodiazepines regarding oxidative stress were also observed in the brain of mice underwent 72h of sleep deprivation [134].

GENETIC STUDIES LINKING OXIDATIVE STRESS AND ANXIETY

Several techniques involving genetics, such as knockout and overexpression of enzymes, gene deletion and

polymorphisms, have been applied to study the relationship between anxiety behavior and parameters related to oxidative stress in humans and rodents. Hovatta and colleagues [3] analyzed the expression of two genes that code for proteins involved in the metabolism of antioxidant defense: glutathione reductase 1 and glyoxalase 1. They noted that local overexpression of these genes had a direct correlation with anxiety-like behavior in the mice brain. Moreover, local inhibition of glyoxalase 1 expression by RNA interference decreased the anxiety-like behavior. These results demonstrate a relationship between enzymes related to oxidative stress with anxiety-related behavior, and the authors hypothesized that glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice. Recently, studies [135, 136] also showed that overexpression the enzyme glyoxalase 1 increased anxiety-like behavior. On the other hand, a negative relationship between anxiety-like behavior and glyoxalase 1 expression was also reported: Krömer *et al*. [137] and Ditzen *et al*. [138] found increased expression of glyoxalase several brain areas and in red blood cells in a low anxiety-related behavioral phenotype. In addition, other study [139] found a decreased gene expression for glyoxalase 1 in a mouse strain with higher anxiety-like behavior.

The glyoxalase 1 is an enzyme of the glyoxalase system, and has an important role in the cellular defenses against glycation [140]. Since oxidative stress leads to formation of advanced glycation end-products (AGEs) [140], and contributes to cytotoxicity and inflammation, one would expect that the a decreased activity of glyoxalase 1 would lead to cellular damage. Therefore, regarding the discrepancies between the results observed in different studies, as reported in the previous paragraph, it is possible that genetic variations or different models (including the oxidative status – high or low oxidative stress – of the animals) could influence the outcome on anxiety-like behavior [135, 141].

Filiou *et al*. [62] combined proteomics and metabolomics approach to analyze mouse models with different levels of anxiety-related behavior, aiming to identify affected pathways for anxiety behavior. They showed increased antioxidant activity and total antioxidant capacity in low anxiety-related behavior mice, and an increased expression of electron transport chain components and adenosine triphosphate synthase in high anxiety-related behavior mice synaptosomes. These data suggest a higher mitochondrial function and possibly higher ROS production in animals that had higher levels of anxiety. Interestingly, the glyoxalase 1 had the most significant difference in expression between high and low anxiety-like behavior mice, corroborating with the above mentioned studies.

A recent study performed with adolescents showed an association of one polymorphism of the nitric oxide synthase 1 (NOS1 ex1f-VNTR) with anxiety state [142]. Nitric oxide synthase (NOS) is an enzyme that transforms arginine into nitric oxide (NO) and citrulline. NO plays a role as a messenger/modulator in various biological processes. However, it is also considered a free radical intermediate, and it can react with oxygen, leading to cellular oxidative damage [143]. In general, studies have shown that NOS1 knockout present behavioral changes involving increased anxiolytic-like phenotype [144, 145]. On the other hand, Wultsch and colleagues [146] showed reduced anxiety-like behavior in NOS1 knockout mice. Therefore, the involvement of the enzyme NOS1 with anxiety it is not clearly determined.

Glutathione (GSH) plays an important role in cellular defense against oxidative stress, since it eliminates in a non-enzymatic way, hydroxyl radicals and singlet oxygen, and serves as a cofactor for enzymes such as glutathione peroxidase and glutathione S-transferases [147]. Chen and colleagues [148] used knockout mouse line lacking the gene encoding glutamate-cysteine ligase modifier subunit (GCLM), the first and the rate-limiting enzyme in the synthesis of GSH. These animals present reduced GSH levels in tissues. In this study, GCLM knockout mice showed to be less anxious or have increased risk-taking behavior in different tests. A previous study also demonstrated that GCLM knockout mice exhibit reduction of anxiety-like behavior in the elevated plus maze and light/dark box tests [149]. Those authors suggested that the absence of neurotoxicity and neurobehavioral deficits in this knockout mouse line could result from redundant antioxidant systems to protect the brain from ROS damage, even when the GSH system is compromised [148]. Although the results obtained from these animals on anxiety-like behavior are consistent, the differences concerning oxidative stress and anxiety-like behavior need to be further investigated.

By analyzing the relationship of antioxidant enzymes activity with anxiety, a study showed that overexpression of mitochondrial CAT, an important antioxidant enzyme, decreased anxiety in mice, even in the absence of oxidative stress, as commented above (see animal models) [41]. In peripheral blood of patients with post-traumatic stress disorder, Zieker and colleagues [150] observed dowregulation of superoxide dismutase and thioredoxin reductase, enzyme involved with thioredoxin system tied to defense against oxidative damage. This decreased availability of antioxidant enzymes may lead an accumulation of reactive species.

Ascorbic acid is an important antioxidant [151]. Mice null for gulono-γ-lactone oxidase (Gulo) are unable to synthesize ascorbic acid, depending on dietary ascorbic acid for survival. Despite increased oxidative stress, the absence of this enzyme in these animals had no effect on anxiety-like behavior [152, 153]. Another protein involved in antioxidants function, the phospholipid transfer protein (PLTP), is widely expressed in the brain, where it appears to function as a transfer factor for α-tocopherol, the main isomer of vitamin E. PLTP knockout mice have increased anxiety-like behavior in elevated plus-maze test [154], showing that a deficiency in a non-enzymatic antioxidant may affect levels of anxiety. In addition, uncoupling protein 2 (UCP2), a mitochondrial protein that reduces oxidative stress [155], is strongly expressed in areas involved in the central regulation of stress and anxiety [156] (please see Anxiety, oxidative stress and mitochondria, above). Using UCP2 deficient mice, Gimsa *et al*. [93] noted that these animals exhibit anxiety behavior in the elevated plus-maze test, and that it is exacerbated by stress (social disruption).

Finally, it is known that oxidative stress has been implicated in the aging process [157]. Deletion of the p66

Shc gene in mice reduces oxidative stress and extended life span [158]. This type of deletion also lead to reduced anxiety-like behavior, compared to the wild-type mice, and this behavior persists as they aged [159].

Thus, several studies have shown a relationship between oxidative stress and emotional responses, particularly anxiety. Genetic manipulation may be useful as an auxiliary tool to discover the pathways related to anxiety, and, in the future, may provide new therapeutic approaches for these disorders.

NEUROTROPHIC FACTORS, OXIDATIVE STRESS AND ANXIETY

How may the induction of oxidative stress affect anxiety? Besides the possibilities raised above, other possibility could be that oxidative stress affects the secretion of neurotrophic factors by neurons in brain structures critical to anxiety-like behavior. Such factors regulate neuronal survival and proliferation, or promote the expansion of dendritic spines [160-162]. One such factor, brain-derived neurotrophic factor (BDNF) is indeed synthesized and secreted by brain structures such as hippocampus, pre-frontal cortex and amygdala [163-165], which are related to anxiety-like behavior [166, 167]. Sub-chronic induction of oxidative stress leads to reduction of BDNF levels and NFκB mediated upregulation of proinflammatory factors [113]. Conversely, the reversion of some of the pharmacologically induced oxidative stress effects appears to have an up-regulation of neurotrophic factors, such as BDNF [168]. It is interesting that physical exercise exert anxiolytic effects, probable by promoting neurotrophins production, as well as anti-inflammatory and antioxidant activity, in key brain regions [for a review see 169].

Pharmacological studies have also related BDNF and anxiety-like behavior. Intrahippocampal injection of BDNF is able to induce an anxiogenic-like activity [170, 171]. In this context, Dalle Molle and colleagues [172] used a translational approach, using both an animal model and studies in humans, and demonstrated that variations in maternal care (which was related to maternal overprotection) are associated with anxiety and increased peripheral BDNF in both rats and humans [172]. However, the authors did not find associations between brain and plasma BDNF levels in their animal model. Therefore, in this model, peripheral BDNF levels do not appear to directly reflect brain BDNF levels [172]. On the other hand, one study using deletion of BDNF/ tropomyosin receptor kinase B (TrkB) described that the specific lack of TrkB signaling in recently generated neurons leads to a remarkably increased anxiety-like behavior [173]. Moreover, other study found that acute or chronic treatment with the benzodiazepine chlordiazepoxide (10 mg/kg) did not alter BDNF levels [174].

Therefore, there are animal studies demonstrating direct [170-172, 175, 176], or inverse correlation between BDNF and anxiety levels [113, 177]. These results would suggest that BDNF may increase or decrease anxiety behavior, depending either on its levels in specific brain structures or on the animal's conditions. In this context, it has been proposed that increased oxidative stress could lead to impairment of some cellular functions, including production of BDNF. Different outcomes (i.e., distinct psychiatric disorders) would result from these effects acting preferentially in particular brain regions [169]. For example, neuroimaging studies, such as positron emission tomography (PET), have related increased anxiety with increased activation of the amygdala and decreased activation of frontal cortex [178].

CONCLUDING REMARKS

The involvement of oxidative stress with anxiety-like behavior has been widely demonstrated [3, 5, 10, 179]. These studies suggest that a therapy specifically targeting at reducing ROS production will possibly have a beneficial effect in overcoming the oxidative stress and anxiety.

However, the neurobiological pathways underlying the effect of oxidative stress on anxiety symptoms are not fully understood. Although the involvement of inflammatory cytokines, neurotrophins, and mitochondrial function has been considered, there is no clear picture of how these changes are related to the anxiety symptoms. The challenge now is to identify oxidative stress mechanisms likely to be involved in the anxiety symptoms induction. Importantly, understanding these pathways could help to clarify the neurobiology of the anxiety disorder and provide tools for new discovery in therapies and preventive strategies.

CONFLICT OF INTEREST

This paper does not present a conflict of interest.

ACKNOWLEDGEMENTS

Financial support: National Research Council of Brazil (CNPq), and PRONEX, CAPES, FAPERGS/CNPq 10/0018.3.

REFERENCES

[1] Gross, C., Hen, R. The developmental origins of anxiety. *Nat. Rev. Neurosci.*, **2004**, *5*(7), 545-52. http://dx.doi.org/10.1038/nrn1429

[2] Weinberger, D.R. Anxiety at the frontier of molecular medicine. *N. Engl. J. Med.*, **2001**, *344*(16), 1247-9. http://dx.doi.org/10.1056/NEJM200104193441612

[3] 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., Barlow, C. Glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice. *Nature*, **2005**, *438*(7068), 662-6. http://dx.doi.org/10.1038/nature04250

[4] Krolow, R., Noschang, C.G., Arcego, D., Andreazza, A.C., Peres, W., Goncalves, C.A., Dalmaz, C. Consumption of a palatable diet by chronically stressed rats prevents effects on anxiety-like behavior but increases oxidative stress in a sex-specific manner. *Appetite*, **2010**, *55*(1), 108-16. http://dx.doi.org/10.1016/j.appet.2010.03.013

[5] Souza, C.G., Moreira, J.D., Siqueira, I.R., Pereira, A.G., Rieger, DK, Souza, D.O., Souza, T.M., Portela, L.V., Perry, M.L. Highly palatable diet consumption increases protein oxidation in rat frontal cortex and anxiety-like behavior. *Life Sci.*, **2007**, *81*(3), 198-203. http://dx.doi.org/10.1016/j.lfs.2007.05.001

[6] Noschang, C.G., Pettenuzzo, L.F., von Pozzer ,T. E., Andreazza, AC., Krolow, R., Fachin, A., Avila, M.C., Arcego, D., Crema, L.M., Diehl, L.A., Goncalvez, C.A., Vendite, D., Dalmaz, C. Sex-specific differences on caffeine consumption and chronic stress-induced anxiety-like behavior and DNA breaks in the hippocampus. *Pharmacol. Biochem. Behav*., **2009**, *94*(1), 63-9. http://dx.doi.org/10.1016/j.pbb.2009.07.007

[7] Halliwell, B. Oxidative stress and neurodegeneration: where are we now? *J. Neurochem*., **2006**, *97*(6), 1634-58. http://dx.doi.org/10.1111/j.1471-4159.2006.03907.x

[8] Jesberger, J.A., Richardson, J.S. Oxygen free radicals and brain dysfunction. *Int. J. Neurosci*., **1991**, *57*(1-2), 1-17. http://dx.doi.org/10.3109/00207459109150342

[9] Weber, G.F. The pathophysiology of reactive oxygen intermediates in the central nervous system. *Med. Hypotheses*., **1994**, *43*(4), 223-30. http://dx.doi.org/10.1016/0306-9877(94)90070-1

[10] Tsaluchidu, S., Cocchi, M., Tonello, L., Puri, B.K. Fatty acids and oxidative stress in psychiatric disorders. *BMC Psychiatry*, **2008**, *8* Suppl 1:S5. http://dx.doi.org/10.1186/1471-244X-8-S1-S5

[11] Dudkina, N.V., Sunderhaus, S., Boekema, E.J., Braun, H.P. The higher level of organization of the oxidative phosphorylation system: mitochondrial supercomplexes. *J. Bioenerg. Biomembr*., **2008**, *40*(5), 419-24. http://dx.doi.org/10.1007/s10863-008-9167-5

[12] Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., Telser, J. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol*., **2007**, *39*(1), 44-84. http://dx.doi.org/10.1016/j.biocel.2006.07.001

[13] Aksenova, M.V., Aksenov, M.Y., Mactutus, C.F., Booze, R.M. Cell culture models of oxidative stress and injury in the central nervous system. *Curr. Neurovasc. Res*., **2005**, *2*(1),73-89. http://dx.doi.org/10.2174/1567202052773463

[14] Gutteridge, J.M., Halliwell, B. Free radicals and antioxidants in the year 2000. A historical look to the future. *Ann. N. Y. Acad. Sci*., **2000**, *899*, 136-47. http://dx.doi.org/10.1111/j.1749-6632.2000.tb06182.x

[15] Kovacheva-Ivanova, S., Bakalova, R., Ribavov, S.R. Immobilization stress enhances lipid peroxidation in the rat lungs. Materials and methods. *Gen. Physiol. Biophys*., **1994**, *13*(6), 469-82. PMID: 7797054

[16] Mitra, R., Vyas, A., Chatterjee, G., Chattarji, S. Chronic-stress induced modulation of different states of anxiety-like behavior in female rats. *Neurosci. Lett.,* **2005**, *383*(3), 278-83. http://dx.doi.org/10.1016/j.neulet.2005.04.037

[17] Oishi, K., Yokoi, M., Maekawa ,S., Sodeyama, C., Shiraishi, T, Kondo, R., Kuriyama, T., Machida, K. Oxidative stress and haematological changes in immobilized rats. *Acta Physiol. Scand*., **1999**, *165*(1), 65-9. PMID: 10072099

[18] Radak, Z., Sasvari, M., Nyakas, C., Kaneko, T., Tahara, S., Ohno, H., Goto, S. Single bout of exercise eliminates the immobilization-induced oxidative stress in rat brain. *Neurochem. Int.*, **2001**, *39*(1), 33-8. http://dx.doi.org/10.1016/S0197-0186(01)00003-1

[19] Esterbauer, H., Schaur, R.J., Zollner, H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic. Biol. Med*., **1991**, *11*(1), 81-128. http://dx.doi.org/10.1016/0891-5849(91)90192-6

[20] Guichardant, M., Valette-Talbi, L., Cavadini, C., Crozier, G., Berger, M. Malondialdehyde measurement in urine. *J. Chromatogr. B. Biomed. Appl*., **1994**, *655*(1), 112-6. http://dx.doi.org/10.1016/0378-4347(94)00030-1

[21] Bulut, M., Selek, S., Gergerlioglu, H.S., Savas, H.A., Yilmaz, H.R., Yuce, M., Ekici, G. Malondialdehyde levels in adult attention-deficit hyperactivity disorder. *J. Psychiatry Neurosci*., **2007**, *32*(6), 435-8. PMID: 18043768

[22] Chakraborty, S., Singh, O.P., Dasgupta, A., Mandal, N., Nath, D.H. Correlation between lipid peroxidation-induced TBARS level and disease severity in obsessive-compulsive disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **2009**, *33*(2), 363-6. http://dx.doi.org/10.1016/j.pnpbp.2009.01.001

[23] Behl, A., Swami, G., Sircar, S.S., Bhatia, M.S., Banerjee, B.D. Relationship of possible stress-related biochemical markers to oxidative/antioxidative status in obsessive-compulsive disorder. *Neuropsychobiology*, **2010**, *61*(4), 210-4. http://dx.doi.org/10.1159/000306591

[24] Ratnakar, S., Banupriya, C., Doureradjou, P., Vivekanandam, S., Srivastava, M.K., Koner, B.C. Evaluation of anxiety, depression and urinary protein excretion among the family caregivers of advanced cancer patients. *Biol. Psychol*., **2008**, *79*(2), 234-8. http://dx.doi.org/10.1016/j.biopsycho.2008.06.001

[25] Arranz, L., Guayerbas, N., De la Fuente, M. Impairment of several immune functions in anxious women. *J. Psychosom. Res*., **2007**, *62*(1), 1-8. http://dx.doi.org/10.1016/j.jpsychores.2006.07.030

[26] Bilici, M., Efe, H., Koroglu, M.A, Uydu, H.A., Bekaroglu, M., Deger, O. Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by antidepressant treatments. *J. Affect Disord*., **2001**,*64*(1), 43-51. http://dx.doi.org/10.1016/S0165-0327(00)00199-3

[27] Galecki, P., Szemraj, J., Bienkiewicz, M., Florkowski, A., Galecka, E. Lipid peroxidation and antioxidant protection in patients during acute depressive episodes and in remission after fluoxetine treatment. *Pharmacol. Rep*., **2009**, *61*(3), 436-47. PMID: 19605942

[28] Kodydkova, J., Vavrova, L., Zeman, M., Jirak, R., Macasek, J., Stankova, B., Tvrzicka, E., Zak, A. Antioxidative enzymes and increased oxidative stress in depressive women. *Clin. Biochem*., **2009**, *42*(13-14), 1368-74. http://dx.doi.org/10.1016/j.clinbiochem.2009.06.006

[29] Khanzode, S.D., Dakhale, G.N., Khanzode, S.S., Saoji, A., Palasodkar, R. Oxidative damage and major depression: the potential antioxidant action of selective serotonin re-uptake inhibitors. *Redox Rep*., **2003**, *8*(6), 365-70. http://dx.doi.org/10.1179/135100003225003393

[30] Ozdemir, E., Cetinkaya, S., Ersan, S., Kucukosman, S., Ersan, E.E. Serum selenium and plasma malondialdehyde levels and antioxidant enzyme activities in patients with obsessive-compulsive disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **2009**, *33*(1), 62-5. http://dx.doi.org/10.1016/j.pnpbp.2008.10.004

[31] Hazneci, E., Karabulut, A.B., Ozturk, C., Batcioglu, K., Dogan, G., Karaca, S., Esrefoglu, M. A comparative study of superoxide dismutase, catalase, and glutathione peroxidase activities and nitrate levels in vitiligo patients. *Int. J. Dermatol*., **2005**, *44*(8), 636-40. http://dx.doi.org/10.1111/j.1365-4632.2004.02027.x

[32] Atmaca, M., Tezcan, E., Kuloglu, M., Ustundag, B., Tunckol, H. Antioxidant enzyme and malondialdehyde values in social phobia before and after citalopram treatment. *Eur. Arch. Psychiatry Clin. Neurosci*., **2004**, *254*(4), 231-5. http://dx.doi.org/10.1007/s00406-004-0484-3

[33] Kuloglu, M., Atmaca, M., Tezcan, E., Gecici, O., Tunckol, H., Ustundag, B. Antioxidant enzyme activities and malondialdehyde levels in patients with obsessive-compulsive disorder. *Neuropsychobiology*, **2002**, *46*(1), 27-32. http://dx.doi.org/10.1159/000063573

[34] Eskiocak, S., Gozen, A.S., Yapar, S.B., Tavas, F., Kilic, A.S., Eskiocak, M. Glutathione and free sulphydryl content of seminal plasma in healthy medical students during and after exam stress. *Hum. Reprod*., **2005**, *20*(9), 2595-600. http://dx.doi.org/10.1093/humrep/dei062

[35] Tezcan, E., Atmaca, M., Kuloglu, M., Ustundag, B. Free radicals in patients with post-traumatic stress disorder. *Eur. Arch. Psychiatry Clin. Neurosci*., **2003**, *253*(2), 89-91. PMID: 12799747

[36] de Oliveira, M.R., Silvestrin, R.B., Mello, E.S.T., Moreira, J.C. Oxidative stress in the hippocampus, anxiety-like behavior and decreased locomotory and exploratory activity of adult rats: effects of sub acute vitamin A supplementation at therapeutic doses. *Neurotoxicology*, **2007**, *28*(6), 1191-9. http://dx.doi.org/10.1016/j.neuro.2007.07.008

[37] Brocardo, P.S., Boehme, F., Patten, A., Cox, A., Gil-Mohapel, J., Christie, B.R. Anxiety- and depression-like behaviors are accompanied by an increase in oxidative stress in a rat model of fetal alcohol spectrum disorders: Protective effects of voluntary physical exercise. *Neuropharmacology,* **2012**, *62*(4), 1607-18. http://dx.doi.org/10.1016/j.neuropharm.2011.10.006

[38] Merzoug, S., Toumi, M.L., Boukhris, N., Baudin, B, Tahraoui, A. Adriamycin-related anxiety-like behavior, brain oxidative stress and myelotoxicity in male Wistar rats. *Pharmacol. Biochem. Behav*., **2011**, *99*(4), 639-47. http://dx.doi.org/10.1016/j.pbb.2011.06.015

[39] Bouayed, J., Rammal, H., Younos, C., Soulimani, R. Positive correlation between peripheral blood granulocyte oxidative status and level of anxiety in mice. *Eur. J. Pharmacol*., **2007**, *564*(1-3), 146-9. http://dx.doi.org/10.1016/j.ejphar.2007.02.055

[40] Rammal, H., Bouayed, J., Younos, C., Soulimani, R. Evidence that oxidative stress is linked to anxiety-related behaviour in mice. *Brain Behav. Immun*., **2008**, *22*(8), 1156-9. http://dx.doi.org/10.1016/j.bbi.2008.06.005

[41] Olsen, R.H., Johnson, L.A., Zuloaga, D.., Limoli, C.L., Raber, J. Enhanced hippocampus-dependent memory and reduced anxiety in mice over-expressing humhttp://dx.doi.org/10.1111/jnc.12187an catalase in mitochondria. *J. Neurochem*., **2013**, Feb 5.

[42] Viggiano, A., Viggiano, E., Monda, M., Ingrosso, D., Perna, A.F., De Luca, B. Methionine-enriched diet decreases hippocampal antioxidant defences and impairs spontaneous behaviour and long-term potentiation in rats. *Brain Res*., **2012**, *1471*, 66-74. http://dx.doi.org/10.1016/j.brainres.2012.06.048

[43] Li, Q., Zhang, M., Chen, Y.J., Wang, Y.J., Huang, F., Liu, J. Oxidative damage and HSP70 expression in masseter muscle induced by psychological stress in rats. *Physiol. Behav*., **2011**, *104*(3), 365-72. http://dx.doi.org/10.1016/j.physbeh.2011.04.012

[44] Noschang, C.G., Krolow, R., Pettenuzzo, L.F, Avila, M.C., Fachin A, Arcego, D., von Pozzer, T. E., Crema, L.M., Diehl, L.A., Vendite, D, Dalmaz, C. Interactions between chronic stress and chronic consumption of caffeine on the enzymatic antioxidant system. *Neurochem. Res*., **2009**, *34*(9), 1568-74. http://dx.doi.org/10.1007/s11064-009-9945-4

[45] Ciobica, A., Hritcu, L., Padurariu, M., Dobrin, R., Bild, V. Effects of serotonin depletion on behavior and neuronal oxidative stress status in rat: relevance for anxiety and affective disorders. *Adv. Med. Sci*., **2010**, *55*(2), 289-96. http://dx.doi.org/10.2478/v10039-010-0035-2

[46] Herman, J.P., McKlveen, J.M., Solomon, M.B., Carvalho-Netto, E., Myers, B. Neural regulation of the stress response: glucocorticoid feedback mechanisms. *Braz. J. Med Biol. Res.*, **2012**, *45*(4), 292-8. http://dx.doi.org/10.1590/S0100-879X2012007500041

[47] Faravelli, C., Lo Sauro, C., Lelli, L., Pietrini, F, Lazzeretti, L, Godini L, Benni, L., Fioravanti, G., Talamba, G.A., Castellini, G., Ricca, V. The role of life events and HPA axis in anxiety disorders: a review. *Curr. Pharm. Des*., **2012**, *18*(35), 5663-74. http://dx.doi.org/10.2174/138161212803530907

[48] Salim, S., Sarraj, N., Taneja, M., Saha, K., Tejada-Simon, M.V., Chugh, G. Moderate treadmill exercise prevents oxidative stress-induced anxiety-like behavior in rats. *Behav. Brain Res*., **2010**, *208*(2), 545-52. http://dx.doi.org/10.1016/j.bbr.2009.12.039

[49] Salim, S., Asghar, M., Chugh, G., Taneja, M., Xia, Z., Saha, K. Oxidative stress: a potential recipe for anxiety, hypertension and insulin resistance. *Brain Res*., **2010**, *1359*, 178-85. http://dx.doi.org/10.1016/j.brainres.2010.08.093

[50] Masood, A., Nadeem, A., Mustafa, S.J., O'Donnell, J.M. Reversal of oxidative stress-induced anxiety by inhibition of phosphodiesterase-2 in mice. *J. Pharmacol. Exp. Ther*., **2008**, *326*(2), 369-79. http://dx.doi.org/10.1124/jpet.108.137208

[51] Nicholls, D.G. Mitochondria and calcium signaling. *Cell Calcium*, **2005**, *38*(3-4), 311-7. http://dx.doi.org/10.1016/j.ceca.2005.06.011

[52] Manji, H.K., Drevets, W.C., Charney, D.S. The cellular neurobiology of depression. *Nat. Med*., **2001**, *7*(5), 541-7. PMID: 11329053

[53] Sapolsky, R.M. The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death. *Biol. Psychiatry*, **2000**, *48*(8), 755-65. http://dx.doi.org/10.1016/S0006-3223(00)00971-9

[54] Schapira, A.H. Mitochondrial diseases. *Lancet*, **2012**, 379(9828), 1825-34. http://dx.doi.org/10.1016/S0140-6736(11)61305-6

[55] Rezin, G.T., Amboni, G., Zugno, A.I., Quevedo, J., Streck, E.L. Mitochondrial dysfunction and psychiatric disorders. *Neurochem. Res*., **2009**, *34*(6), 1021-9. http://dx.doi.org/10.1007/s11064-008-9865-8

[56] Shao, L., Martin, M.V., Watson SJ, Schatzberg, A., Akil, H., Myers, R.M., Jones, E.G., Bunney, W.E., Vawter, M.P. Mitochondrial involvement in psychiatric disorders. *Ann. Med*., **2008**, *40*(4), 281-95. http://dx.doi.org/10.1080/07853890801923753

[57] Jou, S.H, Chiu, N.Y., Liu, C.S. Mitochondrial dysfunction and psychiatric disorders. *Chang Gung Med. J*., **2009**, *32*(4), 370-9. PMID: 19664343

[58] Orth, M., Schapira, A.H. Mitochondria and degenerative disorders. *Am. J. Med. Genet*., **2001**, *106*(1), 27-36. http://dx.doi.org/10.1002/ajmg.1425

[59] Koopman WJ, Nijtmans LG, Dieteren CE, Roestenberg P, Valsecchi F, Smeitink JA, Willems PH. Mammalian mitochondrial complex I: biogenesis, regulation, and reactive oxygen species generation. *Antioxid Redox Signal*., **2010**, *12*(12), 1431-70. http://dx.doi.org/10.1089/ars.2009.2743

[60] Moro, M.A., Almeida, A., Bolanos, J.P., Lizasoain, I. Mitochondrial respiratory chain and free radical generation in stroke. *Free Radic Biol. Med.*, **2005**, *39*(10), 1291-304. http://dx.doi.org/10.1016/j.freeradbiomed.2005.07.010

[61] Rodriguez-Enriquez, S., Kai, Y., Maldonado, E., Currin, R.T., Lemasters, J.J. Roles of mitophagy and the mitochondrial permeability transition in remodeling of cultured rat hepatocytes. *Autophagy*, **2009**, *5*(8), 1099-106. http://dx.doi.org/10.4161/auto.5.8.9825

[62] Filiou, M.D., Zhang, Y., Teplytska, L., Reckow, S., Gormanns, P., Maccarrone, G., Frank, E., Kessler, M.S., Hambsch, B., Nussbaumer M., Bunck, M., Ludwig, T, Yassouridis, A., Holsboer, F., Landgraf, R., Turck, C.W. Proteomics and metabolomics analysis of a trait anxiety mouse model reveals divergent mitochondrial pathways. *Biol. Psychiatry*, **2011**, *70*(11), 1074-82. http://dx.doi.org/10.1016/j.biopsych.2011.06.009

[63] Gould E, Woolley CS, McEwen BS. Short-term glucocorticoid manipulations affect neuronal morphology and survival in the adult dentate gyrus. *Neuroscience*., **1990**, *37*(2), 367-75. http://dx.doi.org/10.1016/0306-4522(90)90407-U

[64] Du, J., Wang, Y., Hunter, R., Wei, Y., Blumenthal, R., Falke, C, Khairova, R., Zhou, R., Yuan, P., Machado-Vieira, R., McEwen, B.S., Manji, H.K. Dynamic regulation of mitochondrial function by glucocorticoids. *Proc. Natl. Acad. Sci. U. S. A*., **2009**, *106*(9), 3543-8. http://dx.doi.org/10.1073/pnas.0812671106

[65] Stein-Behrens, B.A., Elliott, E.M., Miller, C.A., Schilling, J.W., Newcombe, R., Sapolsky, R.M. Glucocorticoids exacerbate kainic acid-induced extracellular accumulation of excitatory amino acids in the rat hippocampus. *J. Neurochem*., **1992**, *58*(5), 1730-5. http://dx.doi.org/10.1111/j.1471-4159.1992.tb10047.x

[66] McEwen, B.S. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol. Rev*., **2007**, 87(3), 873-904. http://dx.doi.org/10.1152/physrev.00041.2006

[67] Simpson, E.R., Waterman, M.R. Regulation by ACTH of steroid hormone biosynthesis in the adrenal cortex. *Can. J. Biochem. Cell Biol*., **1983**, *61*(7), 692-707. http://dx.doi.org/10.1139/o83-088

[68] Jefcoate, C. High-flux mitochondrial cholesterol trafficking, a specialized function of the adrenal cortex. *J. Clin. Invest*., **2002**, *110*(7), 881-90. http://dx.doi.org/10.1172/JCI200216771

[69] Costa, B., Da Pozzo, E., Martini, C. Translocator protein as a promising target for novel anxiolytics. *Curr. Top. Med. Chem*., **2012**, *12*(4), 270-85. http://dx.doi.org/10.2174/156802612799078720

[70] Taliani, S., Da Settimo, F., Da Pozzo, E., Chelli, B., Martini, C. Translocator protein ligands as promising therapeutic tools for anxiety disorders. *Curr. Med. Chem*., **2009**, *16*(26), 3359-80. http://dx.doi.org/10.2174/092986709789057653

[71] Gavish, M., Bachman, I., Shoukrun, R., Katz, Y., Veenman, L., Weisinger, G., Weizman, A. Enigma of the peripheral benzodiazepine receptor. *Pharmacol. Rev*.,**1999**, *51*(4), 629-50. PMID: 10581326

[72] Casellas, P., Galiegue, S., Basile, A.S. Peripheral benzodiazepine receptors and mitochondrial function. *Neurochem. Int*., **2002**, *40*(6), 475-86. http://dx.doi.org/10.1016/S0197-0186(01)00118-8

[73] Corsi, L., Geminiani, E., Baraldi, M. Peripheral benzodiazepine receptor (PBR) new insight in cell proliferation and cell differentiation review. *Curr. Clin. Pharmacol*., **2008**, *3*(1), 38-45. http://dx.doi.org/10.2174/157488408783329878

[74] Veenman, L., Papadopoulos, V., Gavish, M. Channel-like functions of the 18-kDa translocator protein (TSPO): regulation of apoptosis and steroidogenesis as part of the host-defense response. *Curr. Pharm. Des*., **2007**, *13*(23), 2385-405. http://dx.doi.org/10.2174/138161207781368710

[75] Strohle, A., Romeo, E., di Michele, F., Pasini, A., Yassouridis, A., Holsboer, F., Rupprecht, R. GABA(A) receptor-modulating neuroactive steroid composition in patients with panic disorder before and during paroxetine treatment. *Am. J. Psychiatry*, **2002**, *159*(1), 145-7. http://dx.doi.org/10.1176/appi.ajp.159.1.145

[76] Brambilla, F., Biggio, G., Pisu, M.G., Bellodi, L., Perna, G., Bogdanovich-Djukic, V., Purdy, R.H., Serra, M. Neurosteroid secretion in panic disorder. *Psychiatry Res*., **2003**, *118*(2), 107-16. http://dx.doi.org/10.1016/S0165-1781(03)00077-5

[77] Heydari, B., Le Melledo, J.M. Low pregnenolone sulphate plasma concentrations in patients with generalized social phobia. *Psychol. Med*., **2002**, *32*(5), 929-33. http://dx.doi.org/10.1017/S0033291702005238

[78] Rupprecht, R., Papadopoulos, V., Rammes, G., Baghai, T.C., Fan, J., Akula, N., Groyer, G., Adams, D., Schumacher, M. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. *Nat. Rev. Drug Discov*., **2010**, *9*(12), 971-88. http://dx.doi.org/10.1038/nrd3295

[79] Mills, C.D., Bitler, J.L., Woolf, C.J. Role of the peripheral benzodiazepine receptor in sensory neuron regeneration. *Mol. Cell Neurosci*., **2005**, *30*(2), 228-37. http://dx.doi.org/10.1016/j.mcn.2005.07.010

[80] Bordet, T., Buisson, B., Michaud, M., Drouot, C., Galea, P., Delaage, P., Akentieva, N.P., Evers, A.S., Covey, D.F., Ostuni, M.A., Lacapere JJ, Massaad, C., Schumacher, M., Steidl, E.M., Maux, D., Delaage, M., Henderson, C.E., Pruss, R.M. Identification and characterization of cholest-4-en-3-one, oxime (TRO19622), a novel drug candidate for amyotrophic lateral sclerosis. *J. Pharmacol. Exp. Ther*., **2007**, *322*(2), 709-20. http://dx.doi.org/10.1124/jpet.107.123000

[81] Verleye, M., Akwa, Y., Liere, P., Ladurelle, N., Pianos, A., Eychenne, B., Schumacher, M., Gillardin, J.M. The anxiolytic etifoxine activates the peripheral benzodiazepine receptor and increases the neurosteroid levels in rat brain. *Pharmacol. Biochem. Behav*., **2005**, *82*(4), 712-20. http://dx.doi.org/10.1016/j.pbb.2005.11.013

[82] Ferzaz, B., Brault, E., Bourliaud, G., Robert, J.P., Poughon, G., Claustre, Y., Marguet, F., Liere, P., Schumacher, M., Nowicki, J.P., Fournier, J., Marabout, B., Sevrin, M., George, P., Soubrie, P., Benavides, J., Scatton, B. SSR180575 (7-chloro-N,N,5-trimethyl-4-oxo-3-phenyl-3,5-dihydro-4H-pyridazino[4,5-b]indole-1 -acetamide), a peripheral benzodiazepine receptor ligand, promotes neuronal survival and repair. *J. Pharmacol. Exp. Ther*., **2002**, *301*(3), 1067-78. http://dx.doi.org/10.1124/jpet.301.3.1067

[83] Veiga, S., Azcoitia, I., Garcia-Segura, L.M. Ro5-4864, a peripheral benzodiazepine receptor ligand, reduces reactive gliosis and protects hippocampal hilar neurons from kainic acid excitotoxicity. *J. Neurosci. Res*., **2005**, *80*(1), 129-37. http://dx.doi.org/10.1002/jnr.20430

[84] Veiga, S., Carrero, P., Pernia, O., Azcoitia, I., Garcia-Segura, L.M. Translocator protein 18 kDa is involved in the regulation of reactive gliosis. *Glia*, **2007**, *55*(14), 1426-36. http://dx.doi.org/10.1002/glia.20558

[85] Soustiel, J.F., Zaaroor, M., Vlodavsky, E., Veenman, L., Weizman, A., Gavish, M. Neuroprotective effect of Ro5-4864 following brain injury. *Exp. Neurol*., **2008**, *214*(2), 201-8. http://dx.doi.org/10.1016/j.expneurol.2008.08.008

[86] Sarnowska, A., Beresewicz, M., Zablocka, B., Domanska-Janik, K. Diazepam neuroprotection in excitotoxic and oxidative stress involves a mitochondrial mechanism additional to the GABAAR and hypothermic effects. *Neurochem. Int*., **2009**, *55*(1-3), 164-73. http://dx.doi.org/10.1016/j.neuint.2009.01.024

[87] Parker, M.A., Bazan, H.E., Marcheselli, V., Rodriguez de, T. E.B., Bazan, N.G. Platelet-activating factor induces permeability transition and cytochrome c release in isolated brain mitochondria. *J. Neurosci. Res*., **2002**, *69*(1), 39-50. http://dx.doi.org/10.1002/jnr.10235

[88] Papadopoulos, V., Lecanu, L. Translocator protein (18 kDa) TSPO: an emerging therapeutic target in neurotrauma. *Exp. Neurol*., **2009**, *219*(1), 53-7. http://dx.doi.org/10.1016/j.expneurol.2009.04.016

[89] Serra, M., Madau, P., Chessa, M.F., Caddeo, M., Sanna, E., Trapani, G, Franco, M., Liso, G., Purdy, R,H., Barbaccia, M.L., Biggio, G. 2-Phenyl-imidazo[1,2-a]pyridine derivatives as ligands for peripheral benzodiazepine receptors: stimulation of neurosteroid synthesis and anticonflict action in rats. *Br. J. Pharmacol*., **1999**, *127*(1), 177-87. http://dx.doi.org/10.1038/sj.bjp.0702530

[90] Bitran, D., Foley, M., Audette, D., Leslie, N., Frye, C.A. Activation of peripheral mitochondrial benzodiazepine receptors in the hippocampus stimulates allopregnanolone synthesis and produces anxiolytic-like effects in the rat. *Psychopharmacology (Berl)*., **2000**, *151*(1), 64-71. http://dx.doi.org/10.1007/s002130000471

[91] Korneyev, A., Pan, B.S., Polo, A., Romeo, E., Guidotti, A., Costa, E. Stimulation of brain pregnenolone synthesis by mitochondrial diazepam binding inhibitor receptor ligands *in vivo*. *J Neurochem*., **1993**, *61*(4), 1515-24. http://dx.doi.org/10.1111/j.1471-4159.1993.tb13647.x

[92] Romeo, E., Cavallaro, S., Korneyev, A, Kozikowski, A.P., Ma D, Polo, A., Costa, E., Guidotti, A. Stimulation of brain steroidogenesis by 2-aryl-indole-3-acetamide derivatives acting at the mitochondrial diazepam-binding inhibitor receptor complex. *J. Pharmacol. Exp. Ther*., **1993**, *267*(1), 462-71. PMID: 8229777

[93] Gimsa, U., Kanitz, E., Otten, W., Aheng, C., Tuchscherer, M., Ricquier, D., Miroux, B., Ibrahim, S.M. Alterations in anxiety-like behavior following knockout of the uncoupling protein 2 (ucp2) gene in mice. *Life Sci*., **2011**, *89*(19-20), 677-84. http://dx.doi.org/10.1016/j.lfs.2011.08.009

[94] Andrews, Z.B., Rivera, A., Elsworth, J.D., Roth, R.H., Agnati, L., Gago, B., Abizaid, A., Schwartz, M., Fuxe, K., Horvath, T. L. Uncoupling protein-2 promotes nigrostriatal dopamine neuronal function. *Eur. J. Neurosci*., **2006**, *24*(1), 32-6. http://dx.doi.org/10.1111/j.1460-9568.2006.04906.x

[95] Fuxe, K., Rivera, A., Jacobsen, K.X., Hoistad, M., Leo, G., Horvath, T.L., Staines, W., De la Calle, A., Agnati, L.F. Dynamics of volume transmission in the brain. Focus on catecholamine and opioid peptide communication and the role of uncoupling protein 2. *J. Neural. Transm*., **2005**, *112*(1), 65-76. http://dx.doi.org/10.1007/s00702-004-0158-3

[96] Rohleder, N., Joksimovic, L., Wolf, J.M., Kirschbaum, C. Hypocortisolism and increased glucocorticoid sensitivity of pro-Inflammatory cytokine production in Bosnian war refugees with posttraumatic stress disorder. *Biol Psychiatry*., **2004**, *55*(7), 745-51. http://dx.doi.org/10.1007/s00702-004-0158-3

[97] Gill, J., Vythilingam, M., Page, G.G. Low cortisol, high DHEA, and high levels of stimulated TNF-alpha, and IL-6 in women with PTSD. *J. Trauma Stress*, **2008**, *21*(6), 530-9. http://dx.doi.org/10.1002/jts.20372

[98] Gill, J.M., Saligan, L., Woods, S., Page, G. PTSD is associated with an excess of inflammatory immune activities. *Perspect Psychiatr. Care*, **2009**, *45*(4), 262-77. http://dx.doi.org/10.1111/j.1744-6163.2009.00229.x

[99] von Kanel, R., Begre, S., Abbas, C.C, Saner, H., Gander, M.L., Schmid, J.P. Inflammatory biomarkers in patients with posttraumatic stress disorder caused by myocardial infarction and the role of depressive symptoms. *Neuroimmunomodulation*, **2010**, *17*(1), 39-46. http://dx.doi.org/10.1159/000243084

[100] Pace, T.W., Heim, C.M. A short review on the psychoneuroimmunology of posttraumatic stress disorder: from risk factors to medical comorbidities. *Brain Behav. Immun*., **2011**, *25*(1), 6-13. http://dx.doi.org/10.1016/j.bbi.2010.10.003

[101] Denys, D., Fluitman, S., Kavelaars, A., Heijnen, C., Westenberg, H. Decreased TNF-alpha and NK activity in obsessive-compulsive disorder. *Psychoneuroendocrinology*., **2004**, *29*(7), 945-52. http://dx.doi.org/10.1016/j.psyneuen.2003.08.008

[102] Fluitman, S., Denys, D., Vulink, N., Schutters, S., Heijnen, C., Westenberg, H. Lipopolysaccharide-induced cytokine production in obsessive-compulsive disorder and generalized social anxiety disorder. *Psychiatry Res*., **2010**, *178*(2), 313-6. http://dx.doi.org/10.1016/j.psychres.2009.05.008

[103] Konuk, N., Tekin, I.O., Ozturk, U., Atik, L., Atasoy, N., Bektas, S., Erdogan, A. Plasma levels of tumor necrosis factor-alpha and interleukin-6 in obsessive compulsive disorder. *Mediators Inflamm*., **2007**, *2007***,** 65704. 10.1155/2007/65704

[104] Bazan, N.G. Omega-3 fatty acids, pro-inflammatory signaling and neuroprotection. *Curr. Opin. Clin. Nutr. Metab. Care*, **2007,** *10*(2), 136-41. http://dx.doi.org/10.1097/MCO.0b013e32802b7030

[105] Hovatta, I., Juhila, J., Donner, J. Oxidative stress in anxiety and comorbid disorders. *Neurosci. Res*., **2010**, *68*(4), 261-75. http://dx.doi.org/10.1016/j.neures.2010.08.007

[106] Suganuma, H., Arai, Y., Kitamura, Y., Hayashi, M., Okumura, A., Shimizu, T. Maternal docosahexaenoic acid-enriched diet prevents neonatal brain injury. *Neuropathology,* **2010**, *30*(6), 597-605. http://dx.doi.org/10.1111/j.1440-1789.2010.01114.x

[107] Sun, G.Y., Xu, J., Jensen, M.D., Simonyi, A. Phospholipase A2 in the central nervous system: implications for neurodegenerative diseases. *J Lipid Res*., **2004**, *45*(2), 205-13. http://dx.doi.org/10.1111/j.1440-1789.2010.01114.x

[108] O'Donovan, A., Hughes, B.M., Slavich, G.M., Lynch, L., Cronin, M.T., O'Farrelly, C., Malone, K.M. Clinical anxiety, cortisol and interleukin-6: evidence for specificity in emotion-biology relationships. *Brain Behav. Immun*., **2010**, *24*(7), 1074-7. http://dx.doi.org/10.1016/j.bbi.2010.03.003

[109] Guazelli, C.F., Fattori, V., Colombo, B.B., Georgetti, S.R., Vicentini FT, Casagrande, R., Baracat, M.M., Verri, W.A, Jr. Quercetin-loaded microcapsules ameliorate experimental colitis in mice by anti-inflammatory and antioxidant mechanisms. *J. Nat. Prod*., **2013**, *76*(2), 200-8. http://dx.doi.org/10.1021/np300670w

[110] Toumi, M.L., Merzoug, S., Baudin, B., Tahraoui, A. Quercetin alleviates predator stress-induced anxiety-like and brain oxidative

signs in pregnant rats and immune count disturbance in their offspring. *Pharmacol. Biochem. Behav*., **2013**, *107*C, 1-10. http://dx.doi.org/10.1016/j.pbb.2013.03.009

[111] Elks, C.M., Mariappan, N., Haque, M., Guggilam, A., Majid, D.S., Francis, J. Chronic NF-{kappa}B blockade reduces cytosolic and mitochondrial oxidative stress and attenuates renal injury and hypertension in SHR. *Am. J. Physiol. Renal Physiol*., **2009**, *296*(2), F298-305. http://dx.doi.org/10.1152/ajprenal.90628.2008

[112] Mattson, M.P., Camandola, S. NF-kappaB in neuronal plasticity and neurodegenerative disorders. *J. Clin. Invest*., **2001**, *107*(3), 247-54. http://dx.doi.org/10.1172/JCI11916

[113] Salim, S., Asghar, M., Taneja, M., Hovatta, I., Chugh, G., Vollert, C., Vu, A. Potential contribution of oxidative stress and inflammation to anxiety and hypertension. *Brain Res*., **2011**, *1404*, 63-71. http://dx.doi.org/10.1016/j.brainres.2011.06.024

[114] Bluthe, R.M., Dantzer, R., Kelley, K.W. Effects of interleukin-1 receptor antagonist on the behavioral effects of lipopolysaccharide in rat. *Brain Res*., **1992**, *573*(2), 318-20. DOI: http://dx.doi.org/10.1016/j.brainres.2011.06.024

[115] Sakic, B., Szechtman, H., Talangbayan, H., Denburg, S.D., Carbotte, R.M., Denburg JA. Disturbed emotionality in autoimmune MRL-lpr mice. *Physiol. Behav*., **1994**, *56*(3), 609-17. http://dx.doi.org/10.1016/0031-9384(94)90309-3

[116] Schrott, L.M., Crnic, L.S. Increased anxiety behaviors in autoimmune mice. *Behav. Neurosci.*, **1996**, *110*(3), 492-502. http://dx.doi.org/10.1037//0735-7044.110.3.492

[117] Kustova Y, Sei Y, Morse, H.C, Jr., Basile, A.S. The influence of a targeted deletion of the IFNgamma gene on emotional behaviors. *Brain Behav. Immun*., **1998**, *12*(4), 308-24. http://dx.doi.org/10.1006/brbi.1998.0546

[118] Connor, T.J., Leonard, B.E. Depression, stress and immunological activation: the role of cytokines in depressive disorders. *Life Sci*., **1998**, *62*(7), 583-606. DOI: http://dx.doi.org/10.1016/S0024-3205(97)00990-9

[119] Fiore, M., Alleva, E., Probert, L., Kollias, G., Angelucci, F., Aloe, L. Exploratory and displacement behavior in transgenic mice expressing high levels of brain TNF-alpha. *Physiol. Behav*., **1998**, *63*(4), 571-6. http://dx.doi.org/10.1016/S0031-9384(97)00514-3

[120] Boscarino, J.A. Posttraumatic stress disorder and physical illness: results from clinical and epidemiologic studies. *Ann. N.Y. Acad. Sci*., **2004**, *1032*, 141-53. http://dx.doi.org/10.1196/annals.1314.011

[121] Schneiderman, N., Ironson, G., Siegel, S.D. Stress and health: psychological, behavioral, and biological determinants. *Annu. Rev. Clin. Psychol*., **2005**, *1*, 607-28. http://dx.doi.org/10.1146/annurev.clinpsy.1.102803.144141

[122] Zhou, F.L., Zhang, W.G., Wei, Y.C., Xu, K.L., Hui, L.Y., Wang, X.S., Li, M.Z. Impact of comorbid anxiety and depression on quality of life and cellular immunity changes in patients with digestive tract cancers. *World J. Gastroenterol*., **2005**, *11*(15), 2313-8. PMID: 15818744

[123] Godbout, J.P., Glaser, R. Stress-induced immune dysregulation: implications for wound healing, infectious disease and cancer. *J. Neuroimmune Pharmacol*., **2006**, *1*(4), 421-7. http://dx.doi.org/10.1007/s11481-006-9036-0

[124] Pariante, C.M., Miller, A.H. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. *Biol. Psychiatry*, **2001**, *49*(5), 391-404. http://dx.doi.org/10.1007/s11481-006-9036-0

[125] Raison, C.L., Borisov, A.S., Woolwine, B.J., Massung, B., Vogt, G., Miller, A.H. Interferon-alpha effects on diurnal hypothalamic-pituitary-adrenal axis activity: relationship with proinflammatory cytokines and behavior. *Mol. Psychiatry*, **2010**, *15*(5), 535-47. http://dx.doi.org/10.1038/mp.2008.58

[126] Leonard. B.E., Myint, A. The psychoneuroimmunology of depression. *Hum. Psychopharmacol*., **2009**, *24*(3), 165-75. http://dx.doi.org/10.1002/hup.1011

[127] Calsavara, A.C., Rodrigues, D.H., Miranda, A.S., Costa, P.A., Lima, C.X., Vilela, M.C., Rachid, M.A., Teixeira, A.L. Late Anxiety-Like Behavior and Neuroinflammation in Mice Subjected to Sublethal Polymicrobial Sepsis. *Neurotox. Res*., **2012**, *24,* 103-8. http://dx.doi.org/10.1007/s12640-012-9364-1

[128] Chen, J., Song, Y., Yang, J., Zhang, Y., Zhao, P., Zhu, X.J., Su, H.C. The contribution of TNF-alpha in the amygdala to anxiety in mice with persistent inflammatory pain. *Neurosci. Lett*., **2013**, *541*, 275-80. http://dx.doi.org/10.1016/j.neulet.2013.02.005

[129] Mendez-Cuesta, L.A., Marquez-Valadez, B., Perez-De La, C. V., Escobar-Briones, C., Galvan-Arzate, S, Alvarez-Ruiz, Y., Maldonado, P.D., Santana, R.A., Santamaria, A., Carrillo-Mora, P. Diazepam blocks striatal lipid peroxidation and improves stereotyped activity in a rat model of acute stress. *Basic Clin. Pharmacol. Toxicol*., **2011**, *109*(5), 350-6. http://dx.doi.org/10.1111/j.1742-7843.2011.00738.x

[130] Musavi, S., Kakkar, P. Diazepam induced early oxidative changes at the subcellular level in rat brain. *Mol. Cell Biochem*., **1998**, *178*(1-2), 41-6. PMID: 9546579

[131] El-Sokkary, G.H. Melatonin and vitamin C administration ameliorate diazepam-induced oxidative stress and cell proliferation in the liver of rats. *Cell Prolif*., **2008**, *41*(1), 168-76. http://dx.doi.org/10.1111/j.1365-2184.2007.00503.x

[132] Nunez, M.J., Novio, S., Amigo, G., Freire-Garabal, M. The antioxidant potential of alprazolam on the redox status of peripheral blood leukocytes in restraint-stressed mice. *Life Sci*., **2011**, *89*(17-18), 650-4. http://dx.doi.org/10.1016/j.lfs.2011.07.027

[133] Goyal, R., Anil, K. Protective effect of alprazolam in acute immobilization stress-induced certain behavioral and biochemical alterations in mice. *Pharmacol. Rep*., **2007**, *59*(3), 284-90. PMID: 17652828

[134] Singh, A., Kumar, A. Protective effect of alprazolam against sleep deprivation-induced behavior alterations and oxidative damage in mice. *Neurosci. Res*., **2008**, *60*(4), 372-9. http://dx.doi.org/10.1016/j.neures.2007.12.003

[135] Williams, R.t., Lim, J.E., Harr, B., Wing, C., Walters, R., Distler, M.G., Teschke, M., Wu, C., Wiltshire, T., Su, A.I., Sokoloff ,G., Tarantino, L.M., Borevitz, J.O., Palmer, A.A. A common and unstable copy number variant is associated with differences in Glo1 expression and anxiety-like behavior. *PLoS One*, **2009**, *4*(3), e4649. http://dx.doi.org/10.1371/journal.pone.0004649

[136] Distler, M.G., Plant, L.D., Sokoloff, G., Hawk, A.J., Aneas, I., Wuenschell, G.E., Termini, J., Meredith, S.C., Nobrega, M.A, Palmer, A.A. Glyoxalase 1 increases anxiety by reducing GABAA receptor agonist methylglyoxal. *J. Clin. Invest*., **2012**, *122*(6), 2306-15. http://dx.doi.org/10.1172/JCI61319

[137] Kromer, S.A., Kessler, M.S., Milfay, D., Birg, I.N., Bunck, M., Czibere L, Panhuysen, M., Putz, B., Deussing, J.M., Holsboer, F., Landgraf, R., Turck, C.W. Identification of glyoxalase-I as a protein marker in a mouse model of extremes in trait anxiety. *J. Neurosci*., **2005**, *25*(17), 4375-84. http://dx.doi.org/10.1523/JNEUROSCI.0115-05.2005

[138] Ditzen, C., Jastorff, A.M., Kessler, M.S., Bunck, M., Teplytska, L., Erhardt, A., Kromer, S.A., Varadarajulu, J., Targosz, B.S., Sayan-Ayata, E.F., Holsboer, F., Landgraf, R., Turck, C.W. Protein biomarkers in a mouse model of extremes in trait anxiety. *Mol. Cell Proteom*., **2006**, *5*(10), 1914-20. http://dx.doi.org/10.1074/mcp.M600088-MCP200

[139] Szego, E.M., Janaky, T., Szabo, Z., Csorba, A., Kompagne, H., Muller, G., Levay, G., Simor, A., Juhasz, G., Kekesi, K.A. A mouse model of anxiety molecularly characterized by altered protein networks in the brain proteome. *Eur. Neuropsychopharmacol*., **2010**, *20*(2), 96-111. http://dx.doi.org/10.1016/j.euroneuro.2009.11.003

[140] Thornalley, P.J. Dicarbonyl intermediates in the maillard reaction. *Ann. N.Y. Acad. Sci*., **2005**, *1043*, 111-7. http://dx.doi.org/10.1196/annals.1333.014

[141] Bouayed, J., Rammal, H., Soulimani, R. Oxidative stress and anxiety: relationship and cellular pathways. *Oxid. Med. Cell Longev*., **2009**, *2*(2), 63-7. http://dx.doi.org/10.4161/oxim.2.2.7944

[142] Kurrikoff, T., Lesch, K.P., Kiive, E., Konstabel, K., Herterich, S., Veidebaum, T., Reif, A., Harro, J. Association of a functional variant of the nitric oxide synthase 1 gene with personality, anxiety, and depressiveness. *Dev. Psychopathol*., **2012**, *24*(4), 1225-35. http://dx.doi.org/10.1017/S0954579412000661

[143] Pacher, P., Beckman, J.S., Liaudet, L. Nitric oxide and peroxynitrite in health and disease. *Physiol. Rev*., **2007**, *87*(1), 315-424. http://dx.doi.org/10.1152/physrev.00029.2006

[144] Zhang, J., Huang, X.Y., Ye, M.L., Luo, C.X., Wu, H.Y., Hu, Y., Zhou, Q.G., Wu, D.L., Zhu, L.J., Zhu, D.Y. Neuronal nitric oxide synthase alteration accounts for the role of 5-HT1A receptor in modulating anxiety-related behaviors. *J. Neurosci*., **2010**, *30*(7), 2433-41. http://dx.doi.org/10.1523/JNEUROSCI.5880-09.2010

[145] Weitzdoerfer, R., Hoeger, H., Engidawork, E., Engelmann, M., Singewald, N., Lubec, G., Lubec, B. Neuronal nitric oxide synthase knock-out mice show impaired cognitive performance. *Nitric Oxide*, **2004**, *10*(3), 130-40. http://dx.doi.org/10.1523/JNEUROSCI.5880-09.2010

[146] Wultsch, T., Chourbaji, S., Fritzen, S., Kittel, S., Grunblatt, E., Gerlach, M., Gutknecht, L., Chizat, F., Golfier, G., Schmitt, A., Gass, P., Lesch, K.P., Reif, A. Behavioural and expressional phenotyping of nitric oxide synthase-I knockdown animals. *J. Neural. Transm. Suppl*., **2007**, *72*, 69-85. http://dx.doi.org/10.1007/978-3-211-73574-9_10

[147] Dringen, R. Metabolism and functions of glutathione in brain. *Prog. Neurobiol*., **2000**, *62*(6), 649-71. http://dx.doi.org/10.1016/S0301-0082(99)00060-X

[148] Chen, Y., Curran, C.P., Nebert, D.W., Patel, K.V., Williams, M.T., Vorhees C.V. Effect of chronic glutathione deficiency on the behavioral phenotype of Gclm(-/-) knockout mice. *Neurotoxicol. Teratol*., **2012**, *34*(4), 450-7. doi: 10.1016/j.ntt.2012.04.009. 10.1016/j.ntt.2012.04.009.

[149] Steullet, P., Cabungcal, J.H., Kulak, A., Kraftsik, R., Chen, Y., Dalton T.P., Cuenod, M., Do, K.Q. Redox dysregulation affects the ventral but not dorsal hippocampus: impairment of parvalbumin neurons, gamma oscillations, and related behaviors. *J. Neurosci*., **2010**, *30*(7), 2547-58. http://dx.doi.org/10.1523/JNEUROSCI.3857-09.2010

[150] Zieker, J., Zieker, D., Jatzko, A., Dietzsch, J., Nieselt, K., Schmitt, A., Bertsch, T., Fassbender, K., Spanagel, R., Northoff, H., Gebicke-Haerter, P.J. Differential gene expression in peripheral blood of patients suffering from post-traumatic stress disorder. *Mol. Psychiatry*, **2007**, *12*(2), 116-8. http://dx.doi.org/10.1038/sj.mp.4001905

[151] Gren, A., Barbasz, A., Kreczmer, B., Sieprawska, A., Rudolphi-Skorska, E., Filek, M. Protective effect of ascorbic acid after single and repetitive administration of cadmium in Swiss mice. *Toxicol. Mech. Methods*., **2012**, *22*(8), 597-604. http://dx.doi.org/10.3109/15376516.2012.704957

[152] Harrison, F.E., May, J.M., McDonald, M.P. Vitamin C deficiency increases basal exploratory activity but decreases scopolamine-induced activity in APP/PSEN1 transgenic mice. *Pharmacol. Biochem. Behav*., **2010**, *94*(4), 543-52. http://dx.doi.org/10.1016/j.pbb.2009.11.009

[153] Harrison, F.E., Yu, S.S., Van Den, B. K.L., Li, L., May, J.M., McDonald, M.P. Elevated oxidative stress and sensorimotor deficits but normal cognition in mice that cannot synthesize ascorbic acid. *J. Neurochem*., **2008**, *106*(3), 1198-208. http://dx.doi.org/10.1111/j.1471-4159.2008.05469.x

[154] Desrumaux, C., Risold, P.Y., Schroeder, H., Deckert ,V., Masson, D, Athias, A., Laplanche, H., Le Guern, N., Blache, D., Jiang, X.C., Tall AR, Desor, D., Lagrost, L. Phospholipid transfer protein (PLTP) deficiency reduces brain vitamin E content and increases anxiety in mice. *FASEB J*., **2005**, *19*(2), 296-7. http://dx.doi.org/10.1096/fj.04-2400fje

[155] Arsenijevic, D., Onuma, H., Pecqueur, C., Raimbault, S., Manning, B.S., Miroux, B., Couplan, E., Alves-Guerra, M.C., Goubern, M., Surwit, R., Bouillaud, F., Richard, D., Collins, S., Ricquier, D. Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. *Nat. Genet*., **2000**, *26*(4), 435-9. PMID: 11101840

[156] Richard, D., Rivest, R., Huang, Q., Bouillaud, F., Sanchis, D., Champigny, O., Ricquier, D. Distribution of the uncoupling protein 2 mRNA in the mouse brain. *J. Comp. Neurol*., **1998**, *397*(4), 549-60. http://dx.doi.org/10.1002/(SICI)1096-9861(19980810)397:4<549::AID-CNE7>3.0.CO;2-1

[157] Finkel, T., Holbrook, N.J. Oxidants, oxidative stress and the biology of ageing. *Nature*, **2000**, *408*(6809), 239-47. PMID: 11089981.

[158] Trinei, M., Giorgio, M., Cicalese, A., Barozzi, S., Ventura, A., Migliaccio, E., Milia, E., Padura, I.M., Raker, V.A., Maccarana, M., Petronilli, V., Minucci, S., Bernardi, P., Lanfrancone, L., Pelicci, P.G. A p53-p66Shc signalling pathway controls intracellular redox status, levels of oxidation-damaged DNA and oxidative stress-induced apoptosis. *Oncogene*, **2002**, *21*(24), 3872-8. http://dx.doi.org/10.1038/sj.onc.1205513

[159] Berry, A., Capone, F., Giorgio, M., Pelicci, P.G., de Kloet, E.R., Alleva, E., Minghetti, L., Cirulli, F. Deletion of the life span determinant p66Shc prevents age-dependent increases in emotionality and pain sensitivity in mice. *Exp. Gerontol*., **2007**, *42*(1-2), 37-45. http://dx.doi.org/10.1016/j.exger.2006.05.018

[160] Park, H., Poo, M.M. Neurotrophin regulation of neural circuit development and function. *Nat. Rev. Neurosci*., **2013**, *14*(1), 7-23. http://dx.doi.org/10.1038/nrn3379

[161] Cowansage, K.K., LeDoux, J.E., Monfils, M.H. Brain-derived neurotrophic factor: a dynamic gatekeeper of neural plasticity. *Curr. Mol. Pharmacol*., **2010**, *3*(1), 12-29. http://dx.doi.org/10.2174/1874-470211003010012

[162] Schaaf, M.J., Workel, J.O., Lesscher, H.M., Vreugdenhil, E., Oitzl, M.S., de Kloet, E.R. Correlation between hippocampal BDNF mRNA expression and memory performance in senescent rats. *Brain Res*., **2001**, *915*(2), 227-33. http://dx.doi.org/10.1016/S0006-8993(01)02855-4

[163] Gomez-Palacio-Schjetnan, A., Escobar, M.L. Neurotrophins and Synaptic Plasticity. *Curr. Top. Behav. Neurosci*., **2013**, Mar 22. PMID: 10202541

[164] Suri, D., Vaidya, V.A. Glucocorticoid regulation of brain-derived neurotrophic factor: Relevance to hippocampal structural and functional plasticity. *Neuroscience*, **2013**, *239*, 196-213. http://dx.doi.org/10.1016/j.neuroscience.2012.08.065

[165] Gray, J.D., Milner, T.A., McEwen, B.S. Dynamic plasticity: The role of glucocorticoids, brain-derived neurotrophic factor and other trophic factors. *Neuroscience*, **2013**, *239*, 214-27. http://dx.doi.org/10.1016/j.neuroscience.2012.08.034

[166] Montag, C., Reuter, M., Jurkiewicz, M., Markett, S., Panksepp, J. Imaging the structure of the human anxious brain: a review of findings from neuroscientific personality psychology. *Rev. Neurosci*., **2013**, *24*(2), 167-90. http://dx.doi.org/10.1515/revneuro-2012-0085

[167] Fouche, J.P., van Der, W.N.J., Roelofs, K., Stein, D.J. Recent advances in the brain imaging of social anxiety disorder. *Hum. Psychopharmacol*., **2013**, *28*(1), 102-5. http://dx.doi.org/10.1002/hup.2281

[168] Kim, S., Kwon, J. [6]-Shogaol Attenuates Neuronal Apoptosis in Hydrogen Peroxide-Treated Astrocytes Through the Up-Regulation of Neurotrophic Factors. *Phytother. Res*., **2013**, Feb 11. http://dx.doi.org/10.1002/ptr.4946

[169] Moylan, S., Eyre, H.A., Maes, M., Baune, B.T., Jacka, F.N., Berk, M. Exercising the worry away: How inflammation, oxidative and nitrogen stress mediates the beneficial effect of physical activity on anxiety disorder symptoms and behaviours. *Neurosci. Biobehav. Rev*., **2013**, *37*(4), 573-84. http://dx.doi.org/10.1016/j.neubiorev.2013.02.003

[170] Deltheil, T., Tanaka, K., Reperant, C., Hen, R., David, D.J., Gardier, A.M. Synergistic neurochemical and behavioural effects of acute intrahippocampal injection of brain-derived neurotrophic factor and antidepressants in adult mice. *Int. J. Neuropsychopharmacol*., **2009**, *12*(7), 905-15. http://dx.doi.org/10.1097/FBP.0b013e32834ecb14

[171] Casarotto, P.C., de Bortoli, V.C., Zangrossi, H, Jr. Intrahippocampal injection of brain-derived neurotrophic factor increases anxiety-related, but not panic-related defensive responses: involvement of serotonin. *Behav. Pharmacol*., **2012**, *23*(1), 80-8. http://dx.doi.org/10.1097/FBP.0b013e32834ecb14

[172] Dalle, M.R., Portella, A.K., Goldani, M.Z., Kapczinski, F.P., Leistner-Segala, S., Salum, G.A., Manfro, G.G., Silveira, P.P. Associations between parenting behavior and anxiety in a rodent model and a clinical sample: relationship to peripheral BDNF levels. *Transl. Psychiatry*, **2012**, *2*, e195. http://dx.doi.org/10.1038/tp.2012.126

[173] Bergami, M., Rimondini, R., Santi, S., Blum, R., Gotz, M., Canossa, M. Deletion of TrkB in adult progenitors alters newborn neuron integration into hippocampal circuits and increases anxiety-like behavior. *Proc. Natl. Acad. Sci. U. S. A*., **2008**, *105*(40), 15570-5. http://dx.doi.org/10.1073/pnas.0803702105

[174] Balu, D.T., Hoshaw, B.A., Malberg, J.E., Rosenzweig-Lipson, S., Schechter, L.E., Lucki, I. Differential regulation of central BDNF protein levels by antidepressant and non-antidepressant drug treatments. *Brain Res*., **2008**, *1211*, 37-43. http://dx.doi.org/10.1016/j.brainres.2008.03.023

[175] Fuss, J., Ben, A.N.M., Vogt, M.A., Touma, C., Pacifici, P.G., Palme, R., Witzemann, V., Hellweg, R., Gass, P. Voluntary exercise induces anxiety-like behavior in adult C57BL/6J mice

correlating with hippocampal neurogenesis. *Hippocampus*., **2010**, *20*(3), 364-76. http://dx.doi.org/10.1002/hipo.20634

[176] Van H, J.D., Holmes, P.V., Zellner, A.S., Poudevigne, A., Dishman, R.K. Effects of beta-adrenoreceptor blockade during chronic exercise on contextual fear conditioning and mRNA for galanin and brain-derived neurotrophic factor. *Behav Neurosci*., **2004**, *118*(6), 1378-90. http://dx.doi.org/10.1037/0735-7044.118.6.1378

[177] Duman, C.H., Schlesinger, L., Russell, D.S., Duman, R.S. Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice. *Brain Res*., **2008**, *1199*, 148-58. http://dx.doi.org/10.1016/j.brainres.2007.12.047

[178] Tillfors, M., Furmark, T., Marteinsdottir, I., Fischer, H., Pissiota, A., Langstrom, B., Fredrikson, M. Cerebral blood flow in subjects with social phobia during stressful speaking tasks: a PET study. *Am. J. Psychiatry*, **2001**, *158*(8), 1220-6. http://dx.doi.org/10.1176/appi.ajp.158.8.1220

[179] Allam, F., Dao, A.T., Chugh, G., Bohat, R., Jafri, F., Patki, G., Mowrey, C., Asghar, M., Alkadhi, K.A., Salim, S. Grape Powder Supplementation Prevents Oxidative Stress-Induced Anxiety-Like Behavior, Memory Impairment, and High Blood Pressure in Rats. *J Nutr*., **2013**, Apr 17. http://dx.doi.org/10.3945/jn.113.174649

Received: May 15, 2013 Revised: May 15, 2013 Accepted: November 02, 2013