Immunology
The Journal of cells, molecules, systems and technologies

British Society for immunology

IMMUNOLOGY REVIEW ARTICLE

Immune mechanisms linked to depression via oxidative stress and neuroprogression

Nataliia Bakunina, Carmine M. Pariante and Patricia A. Zunszain

Stress, Psychiatry and Immunology Laboratory, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK

doi:10.1111/imm.12443
Received 28 October 2014; revised 21 December 2014; accepted 5 January 2015.
Correspondence: Patricia A. Zunszain, Stress, Psychiatry and Immunology Laboratory, Institute of Psychiatry, Psychology & Neuroscience, King's College London, 125 Coldharbour Lane, The James Black Centre, London SE5 9NU, UK.
Email: patricia.zunszain@kcl.ac.uk
Senior author: Patricia A. Zunszain

Summary

Emerging evidence suggests a significant role for inflammation and oxidative stress as main contributors to the neuroprogression that is observed in major depressive disorder (MDD), where patients show increased inflammatory and oxidative stress biomarkers. The process of neuroprogression includes stage-related neurodegeneration, cell death, reduced neurogenesis, reduced neuronal plasticity and increased autoimmune responses. Oxidative stress is a consequence of the biological imbalance between reactive oxygen species (ROS) and antioxidants, leading to the alteration of biomolecules and the loss of control of the intracellular redox-related signalling pathways. ROS serve as crucial secondary messengers in signal transduction and significantly affect inflammatory pathways by activating nuclear factor-κB and mitogen-activated protein kinase family stress kinases. When present in excess, ROS inflict damage, affecting cellular constituents with the formation of pro-inflammatory molecules, such as malondialdehyde, 4-hydroxynonenal, neoepitopes and damage-associated molecular patterns promoting immune response, and ultimately leading to cell death. The failure of cells to adapt to the changes in redox homeostasis and the subsequent cell death, together with the damage caused by inflammatory mediators, have been considered as major causes of neuroprogression and hence MDD. Both an activated immune–inflammatory system and increased oxidative stress act synergistically, complicating our understanding of the pathogenesis of depression. The cascade of antioxidative and inflammatory events is orchestrated by several transcription factors, with nuclear factor (erythroid-derived 2)-like 2 and nuclear factor-κB having particular relevance to MDD. This review focuses on potential molecular mechanisms through which impaired redox homeostasis and neuroinflammation can affect the neuronal environment and contribute to depression.

Keywords: neuroinflammation; neuroprogression; reactive oxygen species; signal transduction; transcription factors.

Introduction

Although it is well-established that neuroinflammation and oxidative stress have important roles in neurodegenerative diseases and aging, growing evidence suggests their involvement in the pathogenesis of major depressive disorder (MDD).[1–5] MDD is a multifactorial mood disorder that has been proposed to have a neuroprogressive nature[1,6,7] with accelerated cellular aging[8–10] and higher risk of co-morbid somatic age-related diseases.[11,12] Neuroprogression is considered a potentially progressive stage-related process of neurodegeneration that includes apoptosis, reduced neurogenesis, reduced neuronal plasticity and increased autoimmune responses, all of which can be recognized on clinical, structural and biochemical levels in MDD.[1,13] As such, increased oxidative stress markers along with a neuroinflammatory signature have repeatedly been reported in the blood of depressed patients.[14,15] Molecular signs of inflammation, apoptosis and oxidative stress have been identified in post-mortem studies

looking at gene expression profiles in the prefrontal cortex of MDD patients,[16] and the association between depression, oxidative stress and antioxidant status has been reported[17,18] and confirmed in a recent meta-analysis.[19] However, our mechanistic understanding of molecular pathways through which impaired redox homeostasis interacts with the immune–inflammatory system in relation to MDD is not completely understood. Elevated peripheral markers of oxidative damage to lipids, proteins and DNA, as well as low levels of antioxidant compounds such as co-enzyme Q-10, glutathione, ascorbic acid, vitamin E and polyunsaturated fatty acids, are regularly detected in the blood of depressed patients.[20] All of them contribute to the low function of the antioxidative system and to increased levels of oxidative stress, which have been correlated with the severity of depression.[21] Conversely, antidepressants decrease oxidative stress in animal models of chronic stress[22] and in depressed patients.[23–25] We will discuss here the molecular mechanisms that link oxidative stress, inflammation and MDD, starting with a brief description of each.

The sources of reactive oxygen species and their dual role in neural functioning

The brain accounts for more than 20% of the total consumption of oxygen and despite oxygen being essential for neurons, some of its products can be neurotoxic.[26] Reactive oxygen species (ROS) are highly reactive molecules derived from oxygen possessing unpaired electrons that readily oxidize and modify the functions of RNA, DNA, proteins and lipids, with inevitable damage inflicted to neurons, which will be discussed in detail below.[27] Under normal conditions the levels of ROS are balanced by an antioxidative defence system, but when an imbalance between oxidants and antioxidants occurs a state of oxidative stress is reached. Cells in the brain are especially vulnerable to the detrimental effects of oxidative stress because of their high metabolic rate, the abundance of highly peroxidizable substrates and the modest antioxidant levels present.[28] High levels of oxidative stress biomarkers, such as 8-hydroxydeoxyguanosine and malondialdehyde, a by-product of polyunsaturated fatty acid peroxidation and arachidonic acid, indicative of oxidative DNA damage, together with significantly lowered antioxidant enzyme activity, are a feature of MDD.[20]

Interestingly, although ROS have been conventionally considered as toxic by-products of cellular metabolism, they also act as critical secondary messengers and essential elements of fundamental neurobiological processes such as cell growth, proliferation and differentiation,[29] signalling, migration and adhesion,[30] immune responses, biological synthesis, regulation of gene expression[31] and regulated forms of cell death[32] (Table 1). The generation of ROS, within certain physiological levels, is necessary to maintain redox homeostasis in a living organism. ROS are crucial molecules in fighting bacterial agents causing infections.[33] Furthermore, there are data suggesting that oxidative stress is produced as a result of routine adult neurogenesis.[34] Whether ROS are transit by-products of a highly energy-intensive process, or essential signalling molecules for further implementation of the cellular programme, is still not clear.[35] ROS are mainly generated as a result of physiological intracellular metabolism in mitochondria and peroxisomes, involving a variety of cytosolic enzyme systems. Higher levels of oxidative stress result in mitochondrial dysfunction, which further leads to the production of more free radicals and an exacerbation of the cycle of oxidative stress.[26] Of relevance to mood disorders, chronic mild stress, known to be a major trigger of depressive-like behaviour in animals, leads to damage of mitochondrial ultrastructure and function in mouse brain,[36] and MDD has been associated with dysfunctional mitochondria.[37] Interestingly, ROS are also produced as by-products of monoamine oxidase activity, which is vital to the inactivation of the monoaminergic neurotransmitters serotonin, dopamine, noradrenaline and adrenaline, which are involved in the pathophysiology of depression.[26]

Table 1. Effects of reactive oxygen species depending on their levels and biological context

Pro-survival effects	Pro-death effects
Activation of cell survival pathways	Activation of programmed cell death pathways
Fight against environmental pathogens	Damage of biomolecules:
Facilitation of signal transduction	
Cells growth and differentiation control	• proteins – loss of function
Regulation of gene expression	• lipids – cells structural damage, formation of cytotoxic by-products
	• nucleic acids – changes in physiological gene expression, telomere shortening
Activation of Nrf2 transcription factor – rise of antioxidative defence system	Promotion of excessive inflammation

Primary antioxidant defence and inflammatory transcription factors

The cascade of antioxidative and inflammatory events is orchestrated by several transcription factors, and two key ones, widely expressed in the central nervous system, are nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and nuclear factor-κB (NF-κB). Although the full dynamics of the interactions between Nrf2 and NF-κB remain to be resolved, important aspects of each are worth describing.

Nrf2 is the main cellular defence pathway that is activated in different cell types as a result of oxidative stress, leading to increased induction of the target antioxidants and enzymes that defend against apoptosis.[38] Under unstressed conditions Nrf2 is anchored in the cytoplasm through binding to Kelch like-ECH-associated protein 1 (Keap1), which degrades it by ubiquitination (Fig. 1). Upon oxidative stress, Nrf2 travels to the nucleus where it forms a heterodimer with small Maf protein, binds to a DNA promoter antioxidant responsive element (ARE) and initiates transcription of genes that code for proteins that have cytoprotective effects.[39] Nrf2 keeps under control the regulation of DNA damage recognition, repair and removal, as well as the modulation of proteasomes that are responsible for the degradation of damaged or misfiled proteins. It also controls the levels of key regulatory molecules, including protective proteins such as brain-derived neurotrophic factor and the anti-inflammatory interleukin-10 (IL-10).[40] Due to the vast variety of these protective effects, Nrf2 has been suggested as a promising target to counteract ROS-mediated damage in neurodegenerative diseases and depression.[41,42] The broad functions of Nrf2 upon activation by oxidative stress have been shown with primary cortical neurons, where microarray data revealed that Nrf2 is important for the expression of immune and inflammation genes together with growth factors, signalling proteins and neuron-specific genes.[43] Further support for the beneficial role of this transcription factor comes from studies in mice where its deletion resulted in depressive-like behaviour, reduced levels of dopamine and serotonin and increased levels of glutamate in the prefrontal cortex. Interestingly, treatment of these Nrf2 knockout mice with the anti-inflammatory drug rofecoxib reversed their depressive-like behaviour, suggesting the involvement of Nrf2 in the induction of inflammatory cascades.[44] Conversely, inflammation around birth may have long-term detrimental effects on the Nrf2 system. As such, prenatal inflammation of rodents by exposure to lipopolysaccharide resulted in a dysfunctional Nrf2 response in adulthood, indicated by lower levels of glutathione and a decrease in the activity of glutathione synthase.[45]

Activation of NF-κB by ROS and its redox-related targets

The heterodimeric protein NF-κB is involved in the control of a large number of physiological cellular processes, such as immune responses, cellular growth and apoptosis.[46] Under normal conditions it is inactive in cellular

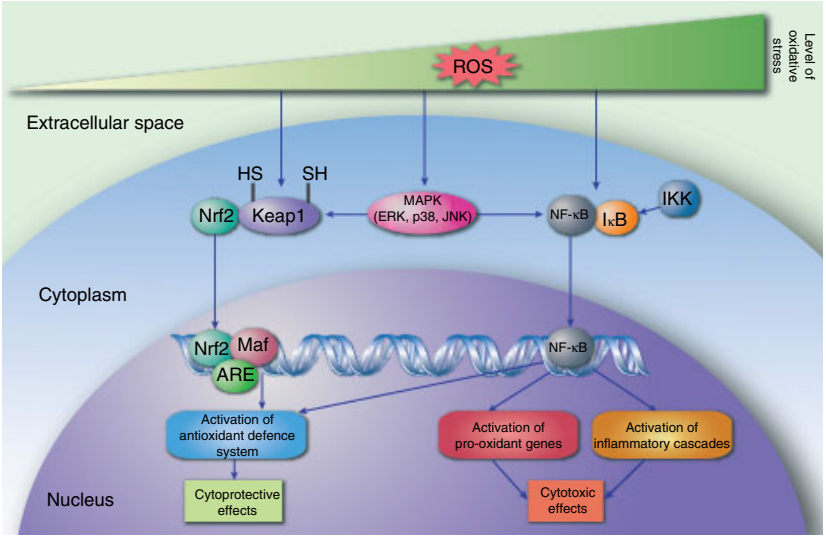

Figure 1. Activation of transcription factors by reactive oxygen species (ROS). ROS can activate different transcription factors directly and/or through kinases. At low levels of oxidative stress, Nrf2 is activated: it is released from the cytoplasm and translocates into the nucleus where it forms a heterodimer with small Maf protein, binds to a DNA promoter Antioxidant Responsive Element (ARE) and initiates transcription of antioxidative genes, leading to cytoprotective effects. At higher levels of oxidative stress, nuclear factor-κB (NF-κB) is acivated: IκB is phosphorylated by IKK and as a result NF-κB is released from the cytoplasm and translocates into the nucleus. Depending on the cellular context, NF-κB can activate inflammatory cascades, pro-oxidant or antioxidant genes.

cytoplasm, where it is bound to the inhibitory protein IκB (Fig. 1). Upon ROS stimulation, IκB is phosphorylated by IKK-kinase and as a result NF-κB is released from the cytoplasm and translocated into the nucleus.[46] In addition to this activation of NF-κB through the classical IKK-dependent pathway, there are other pathways that depend on the cell-type.[47] ROS can activate stress-activated kinases such as extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinases that stimulate NF-κB, which in turn induces the expression of pro-inflammatory cytokines.[48] Furthermore, ROS can modulate NF-κB activity both positively and negatively depending on the context.[49] Whereas through these mechanisms oxidants enhance NF-κB nuclear translocation and therefore its activation, direct oxidation of NF-κB decreases its DNA binding activity and therefore transcription of genes. NF-κB can also target antioxidant genes as well as promote ROS production, which has physiological relevance. For example, activation of JNK by tumour necrosis factor-α requires the generation of ROS, and this process can be counteracted by NF-κB through the induction of genes that encode antioxidizing enzymes such as manganese-superoxide dismutase and ferritin heavy chain.[50,51] Antioxidant and pro-oxidant target genes of NF-κB and their functions are presented in Table 2.[52]

Oxidative stress and neuroinflammation

Regulatory pathways linking inflammation and ROS production are spread beyond transcription factors to proteins and small molecules. Specifically, the generation of ROS is tightly regulated by inflammatory signals, such as the Negative Response ROS (NRROS) protein, highly expressed in immune organs and also detected in the brain. This protein is responsible for the degradation NOX2, one of the membrane-bound subunits of the NADPH oxidase complex, through which a great number of ROS are produced in response to inflammatory stimuli. The NRROS negative regulation of ROS production was shown in interferon-γ and lipopolysaccharide-primed phagocytic cells and in NRROS-knockout mice.[53] Of relevance, down-regulation of NRROS, previously known as Lrrc33 protein, led to increased levels of ROS, whereas knockdown of this enzyme in dendritic cells greatly increased NF-κB activation, even in the absence of any inflammatory stimulation.[54] Hence, chronic inflammation in depressed patients might down-regulate NRROS, therefore resulting in an increase in the levels of ROS and, via a positive feedback loop, a further stimulation of the inflammatory cascade (Fig. 2).

ROS are necessary for the activation of the multiprotein inflammatory complexes known as inflammasomes,[55]

Table 2. Antioxidant and pro-oxidant targets of nuclear factor-κB (NF-κB)

NF-κB targets	Functions
Pro-oxidant NF-κB targets	
NADPH oxidase NOX2 (gp91 phox)	Promotion of reactive oxygen species (ROS) production for immune defences and cell signalling
Xanthine oxidase/dehydrogenase (XOR, or xanthine oxidoreductase)	ROS production through oxidation of hypoxanthine to xanthine
Inducible nitric oxide synthase (iNOS or NOS2)	NO production that further reacts with superoxide leading to formation of the highly reactive peroxynitrite and resultant radicals
Cyclooxygenase-2 (COX-2)	Generation of superoxide during conversion of arachidonic acid into prostaglandin H_2 (PGH_2) by a free radical mechanism
Cytochrome p450 enzymes	Production of ROS when uncoupled, particularly H_2O_2 and hydroxyl radicals
Antioxidant NF-κB targets	
Manganese superoxide dismutase (MnSOD), copper-zinc superoxide dismutase (Cu,Zn-SOD)	Conversion of O_2^- into H_2O_2
Ferritin heavy chain (FHC)	Prevention of iron-mediated generation of highly reactive .OH radicals from H_2O_2
Thioredoxin-1 (Trx1), thioredoxin-2 (Trx2)	Reduction of oxidized proteins
Glutathione S-transferase pi (GST-pi)	Catalysis of the reaction of the GSH thiolate to toxic electrophilic compounds, repair of damage from oxidative stress
Glutathione peroxidase-1 (Gpx1)	Catalysis of the conversion of H_2O_2 into water using glutathione as a substrate, reduction of lipid peroxides and peroxynitrite
Metallothionein-3 (MT3)	Regulation of metal toxicity scavenger of . O_2^- and .OH radicals
NADPH dehydrogenase (quinone) 1 (NQO1)	Prevention of reduction of quinones that produces radical species by its enzymatic activity
Haeme oxygenase 1 (HO-1)	Catalysis of haeme degradation resulting in the formation of carbon monoxide and biliverdin; biliverdin is subsequently reduced to bilirubin, which is a potent antioxidant

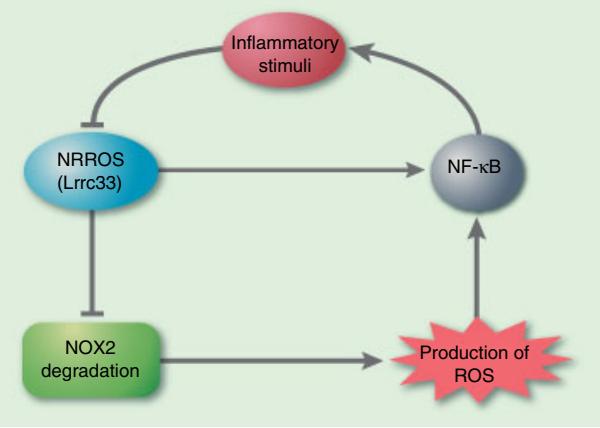

Figure 2. Proposed closed loop of inflammation and reactive oxygen species (ROS) production. Inflammatory stimuli inhibit Negative Response Reactive Oxygen Species (NRROS) protein, in turn inhibiting the degradation of NOX2, which is responsible for ROS production. As a result, more ROS are produced in response to inflammation. ROS in turn activate nuclear factor-κB (NF-κB) and further promote inflammation.

which are a major sensor of cellular stress signals. For example, the NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome, the most extensively studied inflammasome, formed after the oligomerization of NLRP3 and subsequent recruitment of pro-caspase-1, increases IL-1β production and activates NF-κB, further promoting inflammatory cascades.[56–59] ROS change the chemical structures of different molecules, generating a variety of modified oxidation-specific epitopes, which are highly immunogenic and can cause activation of adaptive immunity, such as IgG or IgM-mediated autoimmune responses.[60] For example, significantly greater serum IgG and IgM against oxidized low-density lipoproteins were reported in patients with major depression compared with controls.[61] Furthermore, a separate study in serum of depressed patients detected significant reductions of IgA, which possesses immunomodulatory properties, indicating an attenuation of the anti-inflammatory system in MDD.[62]

As many of the oxidation-specific epitopes have strong pro-inflammatory properties, they are considered as novel kind of damage-associated molecular patterns (DAMPs), discussed below. These molecules can initiate and perpetuate non-infectious immune responses as they bind to cellular and soluble pattern recognition receptors triggering inflammation.[63] Hence, the formation of these epitopes contributes to inflammation and to pathogenic inflammatory pathways postulated to underlie depression, such as tryptophan catabolites produced by the kynurenine pathway.[64] The amino acid tryptophan, a serotonin precursor, is metabolized to kynurenine mainly by indoleamine 2,3-dioxygenase through induction by pro-inflammatory cytokines, such as interferon-γ, tumour necrosis factor-α, IL-2 and IL-1β, and also directly by ROS.[64,65] One of the major products of direct tryptophan oxidation identified *in vivo* is kynurenine.[66] Kynurenine may be further metabolized to excitotoxic free radical generator compounds including 3-hydroxykynurenine and quinolinic acid, whose increased levels, together with increased indoleamine 2,3-dioxygenase activity, have been associated with MDD.[67,68] As a result, oxidative stress contributes to elevated neurotoxic compound via tryptophan oxidation.[66]

The above-mentioned mechanisms might underlie the findings of increased oxidative stress markers and neuroinflammation in depressed patients. We will describe in detail how they potentially contribute to neural cytotoxicity leading to neuroprogression.

Deteriorating effects of ROS and redox-derived inflammatory molecules on neurons

Excessive levels of ROS disrupt the neural cytoarchitecture and affect the function of a variety of biological molecules including lipids, nucleic acids and proteins. As a result some of them undergo modifications and lose anti-inflammatory properties while many more are newly formed, with some possessing pro-inflammatory features.

Lipid peroxidation is particularly important. Polyunsaturated fatty acids, which are more sensitive to oxidation than saturated fatty acids,[69] inhibit prostaglandin E_2 synthesis and modulate immune functions by regulating the production of a variety of cytokines, including IL-1, IL-6, tumour necrosis factor-α and interferon-α.[70] Lower levels of polyunsaturated fatty acids, possibly due to oxidation, have been proposed to contribute to depression pathophysiology. Conversely, polyunsaturated fatty acids have shown protection in subjects at increased risk of developing interferon-α-induced depression, and also against cytokine-induced depressive-like behavioural changes in animal models.[71] Moreover, research has revealed that some molecules generated as a result of lipid peroxidation are recognized by innate immunity and thereby promote inflammatory responses.[63] There are two broad outcomes to lipid peroxidation: structural damage to membranes and generation of oxidation-specific epitopes. These epitopes are cytotoxic secondary products that are formed when lipid hydroperoxides break down in biological systems, such as 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde, both known to be pro-inflammatory.[72,73] These molecules serve as indirect markers of oxidative stress in humans, with increased levels detected in depressed patients. In particular, elevated levels of malondialdehyde, which cause protein damage and generation of advanced lipoxidation end products that also have pro-inflammatory characteristics,[20] have been detected in peripheral blood[74] and serum and plasma[75] of MDD patients. Similarly, other increased markers of lipid

peroxidation serving as DAMPs such as thiobarbituric acid reactive species and 8-iso-prostaglandin-F_{2a} in plasma,[76] have not only been detected but also correlated with the severity of depression.[77]

Nuclear and mitochondrial DNA also suffer from the interaction with ROS, by modification of bases, single- and double-DNA breaks, loss of purines, damage to the deoxyribose sugar, DNA–protein cross-linkage, and damage to the DNA repair system.[78] All of these changes lead to alterations of genetic regulation and can induce programmed cell death. Neurons may respond to unrepaired DNA damage by silencing expression of the affected genomic region, which may be vital for cell survival rather than by undergoing apoptosis.[79] A particular transformation of one of the DNA bases leads to 8-hydroxydeoxyguanosine, which has been widely used as a marker of DNA damage in clinical studies and found to be elevated in depressed patients.[7,80,81] Consistent with previous data showing increased telomere shortening in mood disorders patients,[82] results from a large psychiatric cohort study showed higher levels of telomere shortening among current MDD patients and those in remission, supporting the hypothesis of accelerated cellular aging in depression.[8] Studies demonstrated an exponential correlation between cellular oxidative stress levels and telomere shortening rates, suggesting the significant contribution of oxidative stress-mediated DNA telomere damage as an important determinant of cellular senescent phenotype.[83,84]

Proteins can undergo direct or indirect deterioration following oxidative stress including peroxidation, damage to specific amino acid residues, changes in their tertiary structure, degradation and fragmentation. As a result, proteins lose their enzymic activity, and because of changes in the type and level of cellular proteins, physiological cellular functions are altered.[78] An interesting example is neural cell adhesion molecule (NCAM), a membrane-bound glycoprotein expressed on the surface on neuronal and glial cells. It mediates interactions between different types of neural cells and plays a significant role in fetal and adult neurogenesis, regulating proliferation, differentiation and cell survival. NCAM knockout mice showed behavioural symptoms of depression and attenuated hippocampal neurogenesis,[85] and decreased levels of NCAM were detected after exposure of primary cultured cortical neurons to oxidative stress, which correlated with neuronal death.[86] Importantly, many proteins serve as vital secondary messengers and the functional modifications that proteins can undergo upon oxidation can affect neuronal physiology, which is discussed in the next section.

ROS in signal transduction

Reactive oxygen species cause post-translational protein modifications in a variety of ways including carbonylation, oxidation of aromatic amino acids, methionine sulphoxidation and oxidation of thiol groups on cysteine residues,[87] Among these, the thiol transformations are particularly important because they are reversible and therefore of great physiological relevance in cellular signalling.[88] The most critical to cell fate are transformations of mitogen-activated protein kinases, including ERK, JNK, p38 and BMK1, which are all tightly regulated by ROS. Depending on the site of modification, products of these transformations can inhibit or stimulate kinase activity in a similar way, as happens through phosphorylation.[89,90] Interestingly, ERK, p38 and JNK have been indicated as potential kinases that promote NF-κB and Nrf2 activation. In addition to enzymes from the mitogen-activated protein kinase family, phosphatidylinositide 3-kinases and protein kinase C are also involved in antioxidant regulation. Both of them catalyse phosphorylation of Nrf2, which leads to its dissociation from the Keap 1–Nrf2 complex and causes antioxidant response element-mediated cellular antioxidant responses.[91,92] Importantly, the PI3K/Akt pathway is activated and plays a pleiotropic protective role under oxidative stress through modulation of glycogen synthase kinase 3β, forkhead box-O transcriptional activity and gluthatione metabolism, as shown in mouse hippocampal neurons.[93] In response to oxidative insults, protein kinase pathways are also regulated via phosphorylation of a number of growth factor receptors including the epidermal growth factor receptor, platelet-derived growth factor receptor and the T-cell receptor complex.[94] Whether these pathways are activated or inhibited mostly depends on the cell type and the level of ROS. At higher ROS levels, JNK2 and p38 are activated, stimulating a cell-cycle arrest programme.[90] Under normal conditions the redox regulatory protein thioredoxin has been shown to inhibit the apoptosis signal-regulating kinase 1, involved in activation of JNK and p38. Altered equilibrium of redox homeostasis towards higher levels of ROS causes dissociation of the thioredoxin– apoptosis signal-regulating kinase 1 complex, leading to activation of p38 and JNK.[94] Although there is evidence for both pro-apoptotic and anti-apoptotic properties of p38 under oxidative stress injury, the influence of JNK activation on cell fate is still controversial, because of a lack of efficient pharmacological inhibitors that target this kinase.[94]

Neuronal cell death upon oxidative stress and neuroinflammation

A fundamental process of all neurodegenerative disorders is neuronal cell death. Until recently the most distinct forms of cell death were apoptosis and necrosis, but other forms also affect neurons, and we will describe how oxidative stress and inflammation influence each of them.

Apoptosis is a genetically programmed event, regulated via signalling pathways and high energy demanding,

where organized degradation of the cell occurs within an intact plasma membrane.[95] Its initiation can be implemented through a large number of mechanisms, including activation of stress kinases such as p38 and JNK by ROS. Morphologically apoptotic cells appear shrunken, with dense cytoplasm and tightly packed organelles, pyknotic nucleus, DNA fragmentation and membrane blebs.

Necrosis occurs in response to acute non-physiological stimuli and is associated with cell swelling, gross membrane damage and leakage of cell constituents into the extracellular space. Necrosis leads to the formation of DAMPs, damage of surrounding tissues and local inflammation. Inversely, necrosis can also be a result of general neurotoxicity, where high levels of oxidative stress and inflammation play major roles.[96]

Oxytosis is another form of cell death, more recently described. This is an oxidant-induced cell death with morphological characteristics of necrosis, but which appears to be regulated. It has its own signalling pathways that are independent from classical apoptotic pathways.[97,98] These pathways, yet to be explored, could be potentially targeted therapeutically.

Reactive oxygen species can also cause pyroptosis – another form of cell death with underlying inflammatory mechanisms. In this case ROS activate inflammasomes that lead to caspase-1 activation, IL-1 and IL-18 production, formation of membrane pores, release of these compounds out of the cell and subsequent cell death.[99]

There are ongoing debates about what kind of death cells undergo under various conditions.[100] In rat neuronal cultures it was experimentally demonstrated that ROS could act as initiators or executioners of neuronal death through both necrosis and apoptosis.[101] In primary cultures of murine neocortical neurons following H_2O_2-induced oxidative stress no activation of apoptotic markers, such as caspases, was observed. Instead, autophagic activity was elevated and necrosis features were found, suggesting another combination of molecular mechanisms of neuronal cell death.[102] Further exploration is therefore warranted.

Conclusions and future directions

Despite sufficient evidence linking depression, inflammatory status and oxidative stress, many aspects remain to be explored. Identifying physiological and pathophysiological levels of ROS as well as inflammatory molecules in humans and even across different tissues in the body would allow potential therapeutic strategies directed to maintain physiological levels. Ideally these could be organ and pathology oriented, offering a platform for developing relevant biomarkers and providing a step towards personalized medicine. The measurement of ROS continues to be extremely challenging, especially *in vivo*, because of their short half-life. As ROS functionality and detrimental effects are specific to different cell types, in different context and depending on their levels, further research is necessary in areas of the brain that are known to be affected in depression for delineating specific molecular pathways leading to the progress of the disease. Additionally, a deeper understanding of the interplay between redox status and the immune–inflammatory system would help the identification of the correct targets of neuroprogression to consider in depression. Hence, the ultimate effects of ROS on signal transduction, their interference with inflammatory pathways and their consequences to the cell are mostly determined by the level of these molecules present in the intracellular environment, where higher levels lead to neuroinflammation and cell death. Transcriptional changes of adaptive immunity in MDD, such as activation of NF-κB, can precede inflammatory cascades and oxidative stress, or conversely they can be activated by cytokines and ROS. These changes primarily occur in response to stressful events known to contribute to MDD.[103] One of the features of acquired immunity is the development of immunological memory, which might explain chronic maladaptive alterations leading to a vicious cycle of chronic inflammation and oxidative stress, frequently observed in MDD patients. Furthermore, chronic psychological stress can lead in the long-term to the activation of defence mechanisms that are no longer beneficial but instead accelerate the disease progress.[104]

Interestingly, a recent meta-analysis that evaluated structural brain changes in depressed patients demonstrated reduced hippocampal volume as a consistent finding in those with illness duration of more then 2 years and in those who experienced more then one depressive episode.[105] Although the underlying reasons for these observations are not clear, neuronal cell death is hypothesized to play a major role, where oxidative stress might impose the main damage either directly or through inflammatory pathways. There is plenty of evidence of excessive levels of ROS and inflammatory biomarkers in depressed patients, activation of stress kinases promoting further oxidative stress and neuroinflammation and hence cell death, which might all contribute to neuroprogression and depression. Therefore, investigation of these pathways presents an enormous area for the development of potential therapeutic strategies for depression.

Acknowledgements

The authors received support from the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London, the Commission of European Communities 7th Framework Programme Collaborative Project Grant Agreement n22963 (Mood Inflame) and the Johnson & Johnson-King's College London co-managed

Proof of Concept Fund. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

Disclosures

We declare the following conflicts of interest: CMP has received fees as a speaker or as a member of advisory board, as well as research funding, from pharmaceutical companies that commercialize or are developing antidepressants in the last 3 years, such as Lilly, Servier and Janssen. PAZ has received speaker fees from Servier. NB reports no competing interests.

References

1 Moylan S, Maes M, Wray NR, Berk M. The neuroprogressive nature of major depressive disorder: pathways to disease evolution and resistance, and therapeutic implications. *Mol Psychiatry* 2013; **18**:595–606.

2 Migliore L, Coppedè F. Environmental-induced oxidative stress in neurodegenerative disorders and aging. *Mutat Res* 2009; **674**:73–84.

3 Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature* 2000; **408**:239–47.

4 Shukla V, Mishra SK, Pant HC. Oxidative stress in neurodegeneration. *Adv Pharmacol Sci* 2011; **2011**:13.

5 Li J, O W, Li W, Jiang Z-G, Ghanbari HA. Oxidative stress and neurodegenerative disorders. *Int J Mol Sci* 2013; **14**:24438–75.

6 Myint AM, Kim YK. Cytokine–serotonin interaction through IDO: a neurodegeneration hypothesis of depression. *Med Hypotheses* 2003; **61**:519–25.

7 Maes M, Yirmyia R, Noraberg J *et al.* The inflammatory and neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metab Brain Dis* 2009; **24**:27–53.

8 Verhoeven JE, Revesz D, Epel ES, Lin J, Wolkowitz OM, Penninx BWJH. Major depressive disorder and accelerated cellular aging: results from a large psychiatric cohort study. *Mol Psychiatry* 2014; **19**:895–901.

9 Wolkowitz OW, Epel ES, Reus VI, Mellon SH. Depression gets old fast: do stress and depression accelerate cell aging? *Depress Anxiety* 2010; **27**:327–38.

10 Wolkowitz OM, Reus VI, Mellon SH. Of sound mind and body: depression, disease, and accelerated aging. *Dialogues Clin Neurosci* 2011; **13**:25–39.

11 Gili M, Comas A, García-García M, Monzón S, Antoni S-B, Roca M. Comorbidity between common mental disorders and chronic somatic diseases in primary care patients. *Gen Hosp Psychiatry* 2010; **32**:240–5.

12 Krishnan KRR, Delong M, Kraemer H *et al.* Comorbidity of depression with other medical diseases in the elderly. *Biol Psychiatry* 2002; **52**:559–88.

13 Maes M, Kubera M, Mihaylova I, Geffard M, Galecki P, Leunis J-C, Berk M. Increased autoimmune responses against auto-epitopes modified by oxidative and nitrosative damage in depression: implications for the pathways to chronic depression and neuroprogression. *J Affect Disord* 2013; **149**:23–9.

14 Lopresti AL, Maker GL, Hood SD, Drummond PD. A review of peripheral biomarkers in major depression: the potential of inflammatory and oxidative stress biomarkers. *Prog Neuropsychopharmacol Biol Psychiatry* 2014; **48**:102–11.

15 Maes M, Fišar Z, Medina M, Scapagnini G, Nowak G, Berk M. New drug targets in depression: inflammatory, cell-mediated immune, oxidative and nitrosative stress, mitochondrial, antioxidant, and neuroprogressive pathways. and new drug candidates-Nrf2 activators and GSK-3 inhibitors. *Inflammopharmacology* 2012; **20**:127–50.

16 Shelton RC, Claiborne J, Sidoryk-Wegrzynowicz M, Reddy R, Aschner M, Lewis DA, Mirnics K. Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression. *Mol Psychiatry* 2011; **16**:751–62.

17 Chung CP, Schmidt D, Stein CM, Morrow JD, Salomon RM. Increased oxidative stress in patients with depression and its relationship to treatment. *Psychiatry Res* 2013; **206**:213–6.

18 Stefanescu C, Ciobica A. The relevance of oxidative stress status in first episode and recurrent depression. *J Affect Disord* 2012; **143**:34–8.

19 Palta P, Samuel LJ, Miller ER, Szanton SL. Depression and oxidative stress: results from a meta-analysis of observational studies. *Psychosom Med* 2014; **76**:12–9.

20 Maes M, Galecki P, Chang YS, Berk M. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. *Prog Neuropsychopharmacol Biol Psychiatry* 2011; **35**:676–92.

21 Yanik M, Erel O, Kati M. The relationship between potency of oxidative stress and severity of depression. *Acta Neuropsychiatr* 2004; **16**:200–3.

22 Lee CH, Park JH, Yoo K-Y *et al.* Pre- and post-treatments with escitalopram protect against experimental ischemic neuronal damage via regulation of BDNF expression and oxidative stress. *Exp Neurol* 2011; **229**:450–9.

23 Behr GA, Moreira JCF, Frey BN. Preclinical and clinical evidence of antioxidant effects of antidepressant agents: implications for the pathophysiology of major depressive disorder. *Oxid Med Cell Longev* 2012; **2012**. doi:10.1155/2012/609421.

24 Lee S-Y, Lee S-J, Han C, Patkar AA, Masand PS, Pae C-U. Oxidative/nitrosative stress and antidepressants: targets for novel antidepressants. *Prog Neuropsychopharmacol Biol Psychiatry* 2013; **46**:224–35.

25 Herken H, Gurel A, Selek S *et al.* Adenosine deaminase, nitric oxide, superoxide dismutase, and xanthine oxidase in patients with major depression: impact of antidepressant treatment. *Arch Med Res* 2007; **38**:247–52.

26 Gandhi S, Abramov AY. Mechanism of oxidative stress in neurodegeneration. *Oxid Med Cell Longev* 2012; **2012**:11.

27 Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol* 2003; **552**:335–44.

28 Friedman J. Oxidative stress and free radical damage in neurology. In: Gadoth N, Göbel HH, eds. *Oxidative Stress in Applied Basic Research and Clinical Practice*. Totowa, NJ: Humana Press, 2011:19–27.

29 Sauer H, Wartenberg M, Hescheler J. Reactive oxygen species as intracellular messengers during cell growth and differentiation. *Cell Physiol Biochem* 2001; **11**:173–86.

30 Hurd TR, DeGennaro M, Lehmann R. Redox regulation of cell migration and adhesion. *Trends Cell Biol* 2012; **22**:107–15.

31 Oh JI, Kaplan S. Redox signaling: globalization of gene expression. *EMBO J* 2000; **19**:4237–47.

32 Ray PD, Huang B-W, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal* 2012; **24**:981–90.

33 Nathan C, Cunningham-Bussel A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nat Rev Immunol* 2013; **13**:349–61.

34 Walton NM, Shin R, Tajinda K *et al.* Adult neurogenesis transiently generates oxidative stress. *PLoS One* 2012; **7**:e35264. doi:10.1371/journal.pone.0035264.

35 Woolley JF, Corcoran A, Groeger G, Landry WD, Cotter TG. Redox-regulated growth factor survival signaling. *Antioxid Redox Signal* 2013; **19**:1815–27.

36 Gong Y, Chai Y, Ding J-H, Sun X-L, Hu G. Chronic mild stress damages mitochondrial ultrastructure and function in mouse brain. *Neurosci Lett* 2011; **488**:76–80.

37 Gardner A, Johansson A, Wibom R, Nennesmo I, von Döbeln U, Hagenfeldt L, Hällström T. Alterations of mitochondrial function and correlations with personality traits in selected major depressive disorder patients. *J Affect Disord* 2003; **76**:55–68.

38 Nguyen T, Nioi P, Pickett CB. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *J Biol Chem* 2009; **284**:13291–5.

39 Petri S, Körner S, Kiaei M. Nrf2/ARE signaling pathway: key mediator in oxidative stress and potential therapeutic target in ALS. *Neurol Res Int* 2012; **2012**. doi:10.1155/2012/878030.

40 Baird L, Dinkova-Kostova A. The cytoprotective role of the Keap1–Nrf2 pathway. *Arch Toxicol* 2011; **85**:241–72.

41 de Vries HE, Witte M, Hondius D, Rozemuller AJM, Drukarch B, Hoozemans J, van Horssen J. Nrf2-induced antioxidant protection: a promising target to counteract ROS-mediated damage in neurodegenerative disease? *Free Radic Biol Med* 2008; **45**:1375–83.

42 Calkins MJ, Johnson DA, Townsend JA *et al.* The Nrf2/ARE pathway as a potential therapeutic target in neurodegenerative disease. *Antioxid Redox Signal* 2009; **11**:497–508.

43 Lee J-M, Shih AY, Murphy TH, Johnson JA. NF-E2-related factor-2 mediates neuroprotection against mitochondrial complex I inhibitors and increased concentrations of intracellular calcium in primary cortical neurons. *J Biol Chem* 2003; **278**:37948–56.

44 Martín-de-Saavedra MD, Budni J, Cunha MP *et al.* Nrf2 participates in depressive disorders through an anti-inflammatory mechanism. *Psychoneuroendocrinology* 2013; **38**:2010–22.

45 Zhu Y, Carvey PM, Ling Z. Altered glutathione homeostasis in animals prenatally exposed to lipopolysaccharide. *Neurochem Int* 2007; **50**:671–80.

46 Mohora M, Greabu M, Totan A, Mitrea N. Redox-sensitive signaling factors and antioxidants. *Farmacia* 2009; **57**:399–411.

47 Gloire G, Legrand-Poels S, Piette J. NF-κB activation by reactive oxygen species: fifteen years later. *Biochem Pharmacol* 2006; **72**:1493–505.

48 Rawdin BJ, Mellon SH, Dhabhar FS *et al.* Dysregulated relationship of inflammation and oxidative stress in major depression. *Brain Behav Immun* 2013; **31**:143–52.

49 Siomek A. NF-κB signaling pathway and free radical impact. *Acta Biochim Pol* 2012; **59**:323–31.

50 Nakano H, Nakajima A, Sakon-Komazawa S, Piao J-H, Xue X, Okumura K. Reactive oxygen species mediate crosstalk between NF-κB and JNK. *Cell Death Differ* 2005; **13**:730–7.

51 Perkins ND. Integrating cell-signalling pathways with NF-κB and IKK function. *Nat Rev Mol Cell Biol* 2007; **8**:49–62.

52 Morgan MJ, Liu Z. Crosstalk of reactive oxygen species and NF-κB signaling. *Cell Res* 2011; **21**:103–15.

53 Noubade R, Wong K, Ota N *et al.* NRROS negatively regulates reactive oxygen species during host defence and autoimmunity. *Nature* 2014; **509**:235–9.

54 Liu J, Zhang Z, Chai L, Che Y, Min S, Yang R. Identification and characterization of a unique leucine-rich repeat protein (LRRC33) that inhibits Toll-like receptor-mediated NF-κB activation. *Biochem Biophys Res Commun* 2013; **434**:28–34.

55 Haneklaus M, O'Neill LAJ, Coll RC. Modulatory mechanisms controlling the NLRP3 inflammasome in inflammation: recent developments. *Curr Opin Immunol* 2013; **25**:40–5.

56 Bauer ME, De la Fuente M. Oxidative stress, inflammaging, and immunosenescence. In: Rahman I, Bagchi D, eds. *Inflammation, Advancing Age and Nutrition: Research and Clinical Interventions*. Amsterdam, Netherlands: Elsevier, 2013:39–47.

57 Cruz CM, Rinna A, Forman HJ, Ventura ALM, Persechini PM, Ojcius DM. ATP activates a reactive oxygen species-dependent oxidative stress response and secretion of proinflammatory cytokines in macrophages. *J Biol Chem* 2007; **282**:2871–9.

58 Tschopp J, Schroder K. NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production? *Nat Rev Immunol* 2010; **10**:210–5.

59 Baroja-Mazo A, Martin-Sanchez F, Gomez AI *et al.* The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. *Nat Immunol* 2014; **15**:738–48.

60 Leonard B, Maes M. Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. *Neurosci Biobehav Rev* 2012; **36**:764–85.

61 Maes M, Mihaylova I, Kubera M, Uytterhoeven M, Vrydags N, Bosmans E. Increased plasma peroxides and serum oxidized low density lipoprotein antibodies in major depression: markers that further explain the higher incidence of neurodegeneration and coronary artery disease. *J Affect Disord* 2010; **125**:287–94.

62 Gold PW, Pavlatou MG, Carlson PJ *et al.* Unmedicated, remitted patients with major depression have decreased serum immunoglobulin A. *Neurosci Lett* 2012; **520**:1–5.

63 Weismann D, Binder CJ. The innate immune response to products of phospholipid peroxidation. *Biochim Biophys Acta* 2012; **1818**:2465–75.

64 Maes M, Leonard BE, Myint AM, Kubera M, Verkerk R. The new '5-HT' hypothesis of depression: cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2011; **35**:702–21.

65 Zunszain PA, Anacker C, Cattaneo A *et al.* Interleukin-1β: a new regulator of the kynurenine pathway affecting human hippocampal neurogenesis. *Neuropsychopharmacology* 2012; **37**:939–49.

66 Müller A, Leichert LI. Redox proteomics. In: Jakob U, Reichmann D, eds. *Oxidative Stress and Redox Regulation*. Heidelberg: Springer, 2013:157–86.

67 Miller CL, Llenos IC, Dulay JR, Weis S. Upregulation of the initiating step of the kynurenine pathway in postmortem anterior cingulate cortex from individuals with schizophrenia and bipolar disorder. *Brain Res* 2006; **1073–1074**:25–37.

68 Gabbay V, Klein RG, Katz Y *et al.* The possible role of the kynurenine pathway in adolescent depression with melancholic features. *J Child Psychol Psychiatry* 2010; **51**:935–43.

69 Repetto M, Semprine J, Boveris A. Lipid peroxidation: chemical mechanism, biological implications and analytical determination. In: Catala A, ed. *Lipid Peroxidation*. Croatia: In Tech, 2012.

70 Zunszain PA, Hepgul N, Pariante CM. Inflammation and depression. *Curr Top Behav Neurosci* 2013; **14**:135–51.

71 Su K-P, Huang S-Y, Peng C-Y, Lai H-C, Huang C-L, Chen Y-C, Aitchison KJ, Pariante CM. Phospholipase A2 and cyclooxygenase 2 genes influence the risk of interferon-α-induced depression by regulating polyunsaturated fatty acids levels. *Biol Psychiatry* 2010; **67**:550–7.

72 Catalá A. Lipid peroxidation modifies the picture of membranes from the 'Fluid Mosaic Model' to the 'Lipid Whisker Model'. *Biochimie* 2012; **94**:101–9.

73 Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev* 2014; **2014**. doi: 10.1155/2014/360438.

74 Gałecki P, Szemraj J, Bieńkiewicz M, Florkowski A, Gałecka E. Lipid peroxidation and antioxidant protection in patients during acute depressive episodes and in remission after fluoxetine treatment. *Pharmacol Rep* 2009; **61**:436–47.

75 Sarandol A, Sarandol E, Eker SS, Erdinc S, Vatansever E, Kirli S. Major depressive disorder is accompanied with oxidative stress: short-term antidepressant treatment does not alter oxidative–antioxidative systems. *Hum Psychopharmacol* 2007; **22**:67–73.

76 Dimopoulos N, Piperi C, Psarra V, Lea RW, Kalofoutis A. Increased plasma levels of 8-iso-PGF2α and IL-6 in an elderly population with depression. *Psychiatry Res* 2008; **161**:59–66.

77 Yager S, Forlenza MJ, Miller GE. Depression and oxidative damage to lipids. *Psychoneuroendocrinology* 2010; **35**:1356–62.

78 Kohen R, Nyska A. Invited review: oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol Pathol* 2002; **30**:620–50.

79 Bishop NA, Lu T, Yankner BA. Neural mechanisms of ageing and cognitive decline. *Nature* 2010; **464**:529–35.

80 Forlenza MJ, Miller GE. Increased serum levels of 8-hydroxy-2'-deoxyguanosine in clinical depression. *Psychosom Med* 2006; **68**:1–7.

81 Irie M, Miyata M, Kasai H. Depression and possible cancer risk due to oxidative DNA damage. *J Psychiatr Res* 2005; **39**:553–60.

82 Simon NM, Smoller JW, McNamara KL *et al.* Telomere shortening and mood disorders: preliminary support for a chronic stress model of accelerated aging. *Biol Psychiatry* 2006; **60**:432–5.

83 Richter T, von Zglinicki T. A continuous correlation between oxidative stress and telomere shortening in fibroblasts. *Exp Gerontol* 2007; **42**:1039–42.

84 Kawanishi S, Oikawa S. Mechanism of telomere shortening by oxidative stress. *Ann N Y Acad Sci* 2004; **1019**:278–84.

85 Aonurm-Helm A, Berezin V, Bock E, Zharkovsky A. NCAM-mimetic, FGL peptide, restores disrupted fibroblast growth factor receptor (FGFR) phosphorylation and FGFR mediated signaling in neural cell adhesion molecule (NCAM)-deficient mice. *Brain Res* 2010; **1309**:1–8.

86 Fujita-Hamabe W, Tokuyama S. The involvement of cleavage of neural cell adhesion molecule in neuronal death under oxidative stress conditions in cultured cortical neurons. *Biol Pharm Bull* 2012; **35**:624–8.

87 Bartosz G. Reactive oxygen species: destroyers or messengers? *Biochem Pharmacol* 2009; **77**:1303–15.

88 Forman H, Torres M, Fukuto J. Redox signaling. *Mol Cell Biochem* 2002; **234–235**:49–62.

89 McCubrey JA, LaHair MM, Franklin RA. Reactive oxygen species-induced activation of the MAP kinase signaling pathways. *Antioxid Redox Signal* 2006; **8**:1775–89.

90 Corcoran A, Cotter TG. Redox regulation of protein kinases. *FEBS J* 2013; **280**:1944–65.

91 Buelna-Chontal M, Zazueta C. Redox activation of Nrf2 and NF-κB: a double end sword? *Cell Signal* 2013; **25**:2548–57.

92 Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol* 2004; **55**:373–99.

93 Uranga RM, Katz S, Salvador GA. Enhanced phosphatidylinositol 3-kinase (PI3K)/Akt signaling has pleiotropic targets in hippocampal neurons exposed to iron-induced oxidative stress. *J Biol Chem* 2013; **288**:19773–84.

94 Martindale JL, Holbrook NJ. Cellular response to oxidative stress: signaling for suicide and survival. *J Cell Physiol* 2002; **192**:1–15.

95 Spencer SL, Sorger PK. Measuring and modeling apoptosis in single cells. *Cell* 2011; **144**:926–39.

96 Ryter SW, Kim HP, Hoetzel A, Park JW, Nakahira K, Wang X, Choi AMK. Mechanisms of cell death in oxidative stress. *Antioxid Redox Signal* 2007; **9**:49–89.

97 Tan S. Oxytosis: a novel form of programmed cell death. *Curr Top Med Chem* 2001; **1**:497–506.

98 Henke N, Albrecht P, Bouchachia I *et al.* The plasma membrane channel ORAI1 mediates detrimental calcium influx caused by endogenous oxidative stress. *Cell Death Dis* 2013; **4**:e470.

99 Kepp O, Galluzzi L, Zitvogel L, Kroemer G. Pyroptosis – a cell death modality of its kind? *Eur J Immunol* 2010; **40**:627–30.

100 Vanden Berghe T, Linkermann A, Jouan-Lanhouet S, Walczak H, Vandenabeele P. Regulated necrosis: the expanding network of non-apoptotic cell death pathways. *Nat Rev Mol Cell Biol* 2014; **15**:135–47.

101 Valencia A, Morán J. Reactive oxygen species induce different cell death mechanisms in cultured neurons. *Free Radic Biol Med* 2004; **36**:1112–25.

102 Higgins G, Devenish R, Beart P, Nagley P. Autophagic activity in cortical neurons under acute oxidative stress directly contributes to cell death. *Cell Mol Life Sci* 2011; **68**:3725–40.

103 Bierhaus A, Humpert PM, Nawroth PP. NF-κB as a molecular link between psychosocial stress and organ dysfunction. *Pediatr Nephrol* 2004; **19**:1189–91.

104 Juster R-P, McEwen BS, Lupien SJ. Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neurosci Biobehav Rev* 2010; **35**:2–16.

105 McKinnon MC, Yucel K, Nazarov A, MacQueen GM. A meta-analysis examining clinical predictors of hippocampal volume in patients with major depressive disorder. *J Psychiatry Neurosci* 2009; **34**:41.