ANTIOXIDANTS & REDOX SIGNALING
Volume 9, Number 2, 2007

Forum Review

A Role for Reactive Oxygen/Nitrogen Species and Iron on Neuronal Synaptic Plasticity

CECILIA HIDALGO,[1,2] M. ANGÉLICA CARRASCO,[1,3]
PABLO MUÑOZ,[1,4] and MARCO T. NÚÑEZ[4]

ABSTRACT

A great body of experimental evidence collected over many years indicates that calcium has a central role in a variety of neuronal functions. In particular, calcium participates in synaptic plasticity, a neuronal process presumably correlated with cognitive brain functions such as learning and memory. In contrast, only recently, evidence has begun to emerge supporting a physiological role of reactive oxygen (ROS) and nitrogen (RNS) species in synaptic plasticity. This subject will be the central topic of this review. The authors also present recent results showing that, in hippocampal neurons, ROS/RNS, including ROS generated by iron through the Fenton reaction, stimulate ryanodine receptor-mediated calcium release, and how the resulting calcium signals activate the signaling cascades that lead to the transcription of genes known to participate in synaptic plasticity. They discuss the possible participation of ryanodine receptors jointly stimulated by calcium and ROS/RNS in the normal signaling cascades needed for synaptic plasticity, and how too much ROS production may contribute to neurodegeneration via excessive calcium release. In addition, the dual role of iron as a necessary, but potentially toxic, element for normal neuronal function is discussed. *Antioxid. Redox Signal.* 9, 245–255.

INTRODUCTION

Functionally active neurons display increased oxygen consumption and metabolic activity as well as increased activity-dependent reactive oxygen (ROS) and nitrogen (RNS) species generation. Yet, ROS, and possibly RNS as well, are double-edge swords for neuronal function. On the positive side, ROS/RNS generated during physiological synaptic activity are required for the long-term structural and functional neuronal changes necessary for synaptic plasticity (106). On the negative side, the powerful oxidative metabolism of the brain (25) generates large amounts of ROS as byproducts; this condition increases with age and produces neuronal oxidative stress (26). As a consequence, neuronal survival is at risk during normal aging and in other conditions that promote oxidative stress, including post-traumatic and ischemic conditions or neurodegenerative disorders, such as Alzheimer's and Parkinson disease. Accordingly, a better understanding of the functional relationships between the metabolism of ROS/RNS and normal neuronal functions, including synaptic plasticity, ought to provide novel insights on how to contain the deleterious effects of uncontrolled ROS/RNS production on neuronal survival.

CALCIUM AND SYNAPTIC PLASTICITY

Calcium signals initiate many neuronal responses, including secretion of neurotransmitters, synaptic plasticity, and gene expression (15, 16). Neuronal calcium signals can be produced by calcium influx through plasma membrane voltage- or neurotransmitter-activated calcium channels, or via

[1]Centro FONDAP de Estudios Moleculares de la Célula; [2]Programa de Biología Celular y Molecular; [3]Programa de Fisiología y Biofísica, Instituto de Ciencias Biomédicas, Facultad de Medicina, and [4]Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

calcium release from intracellular stores (15, 84). Depletion of calcium stores also generates calcium influx via store-operated calcium channels (9, 34). Calcium plays a central role in many forms of synaptic plasticity. Long-term potentiation (LTP) and long-term depression (LTD) represent experimental models to study activity-dependent synaptic modifications (12, 78) that cause long-term structural and functional changes in neurons through the expression of new gene products (77). Several steps in the process of activity-dependent gene expression are calcium dependent (85, 113), engaging nuclear and cytoplasmic factors that decode spatial and temporal properties of cellular calcium signals (15, 18, 28, 108).

In the hippocampus, the most extensively studied forms LTP and LTD at the CA1 region depend on *N*-methyl-D-aspartate (NMDA) receptor activation and require a postsynaptic calcium increase (12, 77). Phosphorylation of the nuclear transcription factor cAMP/calcium response element binding protein (CREB) is considered critical for the maintenance of LTP and for several forms of learning and memory (21, 77). The acute expression of constitutively active CREB causes an enhancement of both NMDA receptor-mediated synaptic responses and LTP in the hippocampus (80). Activity-dependent CREB phosphorylation induces the transcription of many neuronal genes, including c-fos and brain-derived neurotrophic factor (BDNF) (86, 113). C-fos is a classical early immediate gene induced by neuronal activity (113) that has been implicated in hippocampal-dependent LTP and memory formation (38). CREB activation may also participate in the activation to egr-1, an early immediate gene induced during many forms of neuronal plasticity in the hippocampus and other brain regions (77). The gene promoters for both c-fos and egr-1 contain response elements for CREB and for serum response factor (SRF), which in hippocampal cells are calcium dependent (56).

CREB-dependent transcription of genes involved in synaptic plasticity entails long-term CREB phosphorylation by the calcium-sensitive Ras/ERK (extracellular signal-regulated kinase) pathway (2, 46, 55, 77, 112, 114). ERK activation is involved in the generation of several forms of LTP and in some types of long-term memory acquisition, such as hippocampus-dependent spatial learning (112). A role for ERK at the level of translational control of gene expression in some forms of hippocampal LTP and memory has also been reported (63).

SYNAPTIC PLASTICITY AND ROS/RNS

A number of cellular enzymes, including nitric oxide synthase, xanthine oxidase, and NADPH oxidase (NOX), as well as the metabolism of arachidonic acid and the mitochondrial electron transport chain, produce RNS/ROS such as nitric oxide (NO), superoxide anion, and hydrogen peroxide (H_2O_2) (30). In addition, the highly reactive hydroxyl radical, which is not produced by any known enzymatic reaction, is formed from H_2O_2 via the Fenton reaction in the presence of redox-active metals such as Fe^{2+}, Mn^{2+}, and Cu^{1+} (109).

Sources of ROS/RNS in the hippocampus

Several reports indicate that NMDA receptor activation promotes ROS and RNS generation in hippocampal neurons (17, 24, 53, 102). Local calcium entry into postsynaptic terminals following NMDA receptor activation stimulates the neuronal NO synthase attached to the postsynaptic membrane complex, leading to significant NO production (48). NMDA receptor stimulation also activates mitochondrial generation of superoxide anion in rat hippocampal pyramidal neurons in culture and in rat brain hippocampal slices (17, 53). Furthermore, hippocampal neurons possess an intrinsic postsynaptic NOX activity that produces superoxide anion following NMDA receptor activation (65, 107, 110). Through enzymatic or chemical dismutation, superoxide anion generates H_2O_2, which represents quantitatively the most important readily permeable peroxide generated by neuronal cells (29). As described above, in the presence of transition metals such as iron, H_2O_2 is converted into the highly reactive hydroxyl radical. We will discuss next possible effects of NO, superoxide anion, H_2O_2, iron, and hydroxyl radical on synaptic plasticity, centering the discussion on their effects on hippocampal neurons.

LTP and reactive nitrogen and oxygen species

Activity-dependent NO generation has been associated with synaptic plasticity and calcium signaling in neurons (99, 116). NO gas is a freely diffusible second messenger that may activate presynaptic or postsynaptic signal transduction pathways, such as the cascade composed of guanylyl cyclase, cGMP-dependent protein kinase, and ADP-ribosylcyclase. Stimulation of ADP-ribosylcyclase enhances in turn the synthesis of cADPR, a second messenger known to activate RyR-mediated calcium release (39, 43). It has also been reported that NO activates a presynaptic component of early LTP, presumably via stimulation of guanylyl cyclase and cGMP-dependent protein kinase (48). In CA1 area neurons, blockade of postsynaptic RyR markedly reduces NO-induced LTP (76), while blockade of RyR—presumably presynaptic—markedly reduces NO-induced LTD (100). Possible mechanisms accounting for RyR involvement in NO-induced LTP will be discussed below.

In addition to NO, ROS have also been implicated in hippocampal LTP (106). Cell-permeable scavengers of superoxide anion block LTP induction in area CA1 of the hippocampus (66). Activation of NMDA receptors stimulates a postsynaptic NOX activity that generates superoxide anion, which readily dismutates into H_2O_2; addition of catalase to scavenge H_2O_2 attenuates LTP in the hippocampus (111), implicating H_2O_2 in LTP induction. Yet, divergent results on the effects of H_2O_2 on hippocampal function have been reported (60). Electrophysiological studies in rat hippocampal slices indicate that H_2O_2 inhibits population spikes (8) and slow onset (NMDA-independent) LTP induced by muscarinic agonists or tetanic stimulation (7). In CA1 in hippocampal slices, initial augmentation and subsequent long-lasting depression of population spikes and excitatory postsynaptic potentials by H_2O_2 have also been reported (62). In contrast, low concentrations of H_2O_2 (1 μM) cause a twofold increase in tetanic LTP and enhance NMDA receptor-independent LTP in the hippocampus compared to controls (59). Likewise, a significant increase in excitatory postsynaptic potentials by H_2O_2 occurs in sympathetic pre-

ganglionic neurons (74). The use in some of these studies of high (m*M*) H_2O_2 concentrations, which are unlikely to occur under physiological conditions and which may promote deleterious oxidative reactions, may explain the reported inhibitory effects of H_2O_2 on LTP (60).

ROS-induced activation of ERK and CREB phosphorylation and early genes in neurons

Recent evidence indicates that ROS have an important role in ERK activation and in long-lasting LTP induction in the hippocampus (65, 106). Pharmacological and genetic manipulations that lead to NOX inhibition abolish NMDA receptor-induced ERK activation (65). These results suggest that NOX-dependent ROS production forms part of the signaling cascades linking stimulation of NMDA receptors with ERK activation in hippocampal neurons. Several reports indicate that exogenously added H_2O_2 also increases ERK phosphorylation in PC12 cells (14, 45, 118, 120) and cortical neurons (27). Treatment of PC12 cells in culture with μ*M* concentrations of H_2O_2 enhances ERK1/2 phosphorylation within minutes (45, 120). In PC12 cells, activation of ERK1/2 phosphorylation by 300 μ*M* H_2O_2 takes place even in the presence of a hydroxyl radical scavenger (118), presumably ruling out Fenton-generated hydroxyl radicals in this response. Stimulation of cortical neurons with 0.1–1 m*M* H_2O_2 for 15 min produces concentration-dependent increases in ERK1/2 and CREB phosphorylation (27). Likewise, PC12 cells exposed to 1 m*M* H_2O_2 show increased CREB phosphorylation (14). In hippocampal slices, 10 m*M* H_2O_2 increases ERK1/2 phosphorylation and this increase is blocked by the antioxidant *N*-acetylcysteine (61).

We have reported recently that H_2O_2 generates intracellular ryanodine-sensitive calcium signals that enhance sequentially ERK and CREB phosphorylation in N2a cells and hippocampal neurons (22, 65). These findings strongly suggest that H_2O_2 stimulates RyR-mediated calcium release from intracellular neuronal stores, as it does in skeletal and cardiac muscle (19, 94) and in RyR-enriched vesicles isolated from these tissues (52, 104). The ensuing calcium concentration increase would promote ERK activation, which in turn would stimulate CREB phosphorylation. Although H_2O_2 can also stimulate ERK through direct redox modifications of the Ras protein (1, 50), we found that preincubation of neurons with ryanodine, in conditions that ensure selective RyR inhibition, prevents the stimulation of ERK/CREB phosphorylation induced by H_2O_2 (Fig. 1). In the same model of hippocampal cells in culture, we have also found that H_2O_2 increased the mRNA levels of the early genes c-fos and egr-1, while preincubation with ryanodine prevented this stimulation (Fig. 2). As discussed above, the promoters for both early immediate genes contain response elements for the transcription factors CREB and SRF, which in hippocampal cells are calcium dependent (56). These results provide further evidence for a link between H_2O_2 and RyR-induced calcium release, and strongly suggest that RyR are primary targets of H_2O_2 in hippocampal neurons. The possible role of H_2O_2-induced RyR stimulation on synaptic plasticity will be discussed below.

A ROLE FOR IRON IN SYNAPTIC PLASTICITY

Iron is a trace element essential to the maintenance of normal physiological functions. In vertebrates, iron-containing proteins play a key role in diverse physiological processes, such as oxygen transport, respiration, DNA synthesis, certain aspects of host defense, xenobiotic metabolism, and the synthesis of some essential neurotransmitters and hormones (44). It has been well established that humans require an adequate iron supply for optimal growth and cognitive development (13); consequently, iron deficiency represents a serious nutrition quandary.

Physiological functions of iron in the brain

Iron has particularly relevant roles in neuronal function. In humans, iron deficiency anemia during infancy is associated with inferior performance on mental and motor tests and on behavioral conduct (32, 42, 75). Animal studies have revealed that feeding rats with low iron diets early in life results in irreversible alterations of brain functions, which are related to insufficient myelination (13, 96), and causes defective establishment of dopaminergic tracts (3, 83). Despite normalization of hematology, growth, and most brain functions, early iron-deficient animals carry persistent deficits in sensory and motor abilities, and in their response to novel settings and performance on spatial learning tasks; all these defects are consistent with fundamental alterations of the striatal dopaminergic and hippocampal systems (36).

Iron as a generator of neuronal ROS

Because of its capacity to participate in one-electron reactions, iron is a pro-oxidant element. The pro-oxidant activity of iron is not necessarily deleterious to neurons, and may even be essential if maintained within physiological limits. Thus, SHSY5Y neuroblastoma cells with decreased iron content exhibit altered excitability, including a reduction in resting membrane potential and in whole cell current amplitude evoked by depolarizing voltage pulses (90). One possible explanation to account for iron essentiality could be its contribution to maintain a necessary "oxidative tone" in neurons. Yet, because of its ability to undergo one-electron reactions, Fe^{2+} catalyzes through the Fenton reaction the transformation of the mild oxidant H_2O_2 into hydroxyl radical, one of the most reactive species in nature (109). The Fenton reaction follows mass action law, so hydroxyl radical production is proportional to the reactive Fe^{2+} concentration. There are no known specific mechanisms to detoxify hydroxyl radical; hence, once generated this species reacts quickly with cellular lipids, proteins, and DNA (44, 47). To maintain iron within a concentration window that allows for its necessary physiological functions and impedes the formation of highly reactive ROS, mammalian cells (including neurons) possess a post-transcriptional regulation mechanism known as the iron responsive element/iron regulatory protein (IRE/IRP) homeostatic system. The IRE/IRP system is a translational regulation system that upon activation by low cellular iron levels induces the expression of transferrin

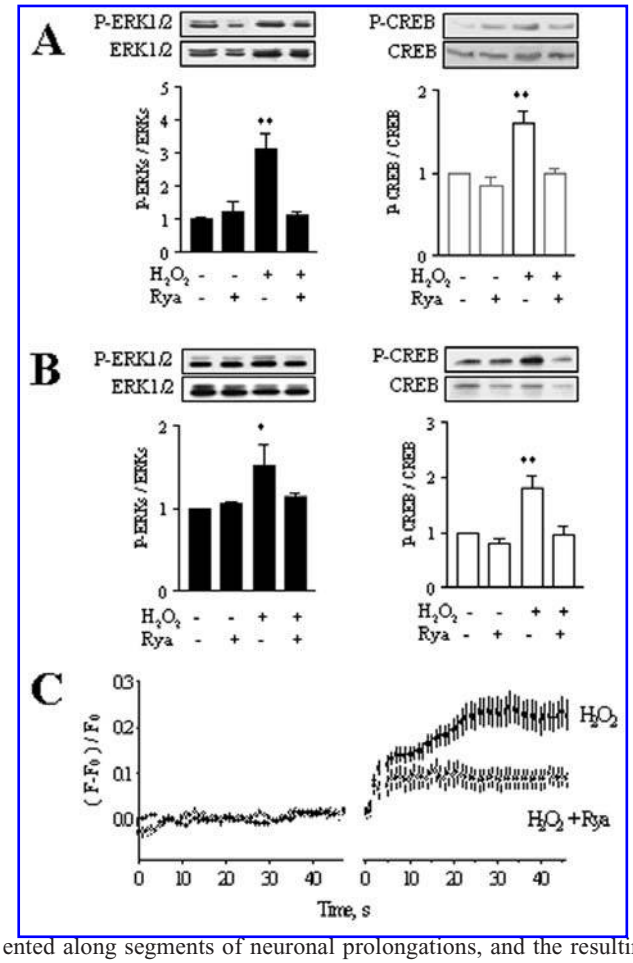

FIG. 1. Effects of H_2O_2 and ryanodine on ERK/CREB phosphorylation (A, B) and cytoplasmic Ca^{2+}signals (C) in hippocampal neurons. (**A**) Hippocampal cells in culture obtained from Sprague–Dawley rats at embryonic day 18 were used for experiments at 12 DIV. The animal ethics committees of the institutions where this work was carried out approved all experimental procedures involving the use of animals. Cells were washed with phosphate buffered saline (PBS) and maintained for 1 h under resting conditions in Krebs–Ringer (in m*M*: 20 HEPES-Tris, pH 7.4, 118 NaCl, 4.7 KCl, 3 $CaCl_2$, 1.2 $MgCl_2$ and 10 glucose) in the absence or presence of 50 μ*M* ryanodine to inhibit RyR. For H_2O_2 stimulation, cells were exposed to 200 μ*M* H_2O_2 for 20 min. (**B**) Hippocampi from 6- to 8-week-old C57/Bl6 male mice were removed, and 400 μm slices were prepared. Slices were maintained in artificial cerebrospinal fluid (in m*M*: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 25 D-glucose, 2 $CaCl_2$, and 1 $MgCl_2$, saturated with 95% O_2/5% CO_2) for 1.5 h before stimulation with H_2O_2. To inhibit RyR, slices were preincubated for 60 min with 50 μ*M* ryanodine before addition of 1 μ*M* H_2O_2. The *upper part* of each panel in **A** or **B** illustrates Western blots for phospho-ERK1/2 or phospho-CREB and for total ERK or CREB as loading controls. The *bar graphs* under the Western blots represent the ratios (mean ± SE) of phospho-ERK1/2 over total ERK (*black solid bars*) or phospho-CREB over total CREB (*gray solid bars*). All values were normalized to the control values obtained in the absence of H_2O_2 or ryanodine. *$p < 0.05$; **$p < 0.01$, ANOVA followed by Dunnett's posttest. (**C**) Intracellular Ca^{2+} signals in hippocampal cells in culture stimulated by H_2O_2. Control hippocampal cells, or cells pretreated with 50 μ*M* ryanodine for 1 h, were loaded with the Ca^{2+} indicator Fluo 3-AM and then exposed to 200 μ*M* H_2O_2. Fluorescence image data were taken at room temperature (20–22ºC) in the time line scan mode in a confocal microscope (Carl Zeiss LSM 5 Pascal, Oberkochen, Germany). Line scans were oriented along segments of neuronal prolongations, and the resulting Ca^{2+} signals are presented as $\Delta F/F_o$ values, where F_o corresponds to the basal fluorescence obtained from 2,000 or 4,000 line scans. Values were obtained from five different cultures, with $n = 23$ for control cells (*closed circles*) and $n = 8$ for cells preincubated with 50 μ*M* ryanodine (*open circles*). All values are given as mean ± SE.

receptor and, probably, of the iron import transporter DMT1 (49, 54). The IRE/IRP system is also activated by oxidative stress, including iron-induced oxidative stress, leading to faulty regulation of iron homeostasis (92).

Although iron seems to be essential for normal brain function, iron accumulation is a source of ROS-mediated cell damage (Fig. 3). Accompanying iron-induced ROS increase, there is a reduction in the reduced glutathione (GSH) content in SHSY5Y cells, which results in a decrease in the reduction potential given by the GSH/GSSG (oxidized glutathione) ratio. Massive cell death correlates with changes in cellular reduction potential, to values more positive than −300 mV (91). Interestingly, even under deleterious iron loads, a fraction of the cell population adapts and survives by significantly increasing their GSH content (4). Thus, iron is a two-faced element, both essential and potentially toxic to neuronal cells. Toxicity arises from the failure of these cells to stop iron accumulation through the IRE/IRP system, leading to the establishment of the vicious cycle "**iron → oxidative stress → IRP1 activity**" (93).

Increasing intracellular iron levels induces RyR-mediated Ca^{2+} release

As described above, in hippocampal synapses a rise in intracellular postsynaptic calcium concentration is required for synaptic plasticity. This increase is initially produced by calcium influx through activated NMDA receptors. Through calcium-induced calcium release (CICR), ryanodine-sensitive intracellular stores contribute to amplify the initial calcium entry signal; the resulting calcium signal triggers the activation of a number of signaling cascades such as the Ras/ERK pathway (69). As already mentioned, recent evidence suggests that ROS participate as second messengers in normal physiological processes in neurons. For example, activation of NMDA receptors results in the production of ROS, which appears to be critical for synaptic plasticity, one of the cellular mechanisms that underlie learning and memory (10, 58). Recent work in our laboratory indicates a novel correlation between iron and calcium signals. Thus, iron addition to PC12 cells (Fig. 4A), or cultured hippocampal neurons (not

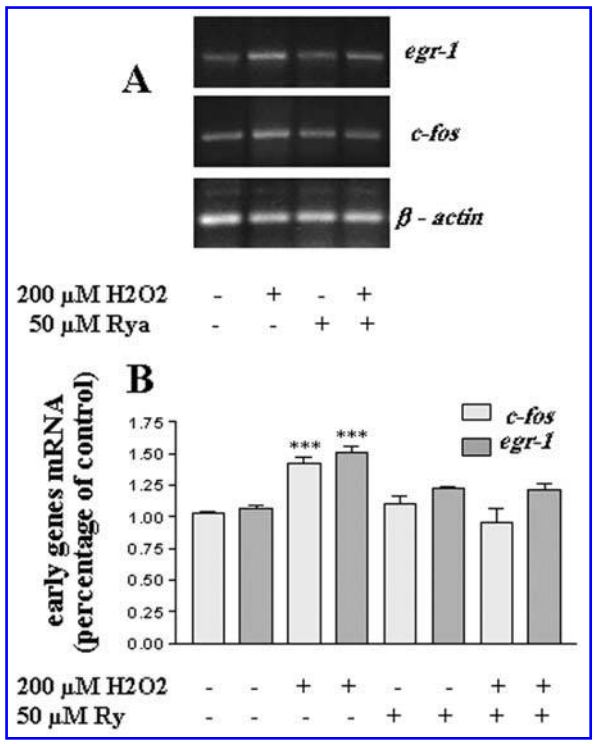

FIG. 2. Effects of H_2O_2 and ryanodine on the mRNA levels of the early immediate genes c-fos and egr-1 in hippocampal cells in culture. Hippocampal neurons in culture were washed with PBS and maintained for 60 min under resting conditions in Krebs–Ringer medium in the presence or absence of 50 µ*M* ryanodine. Cells were exposed for 30 min to 200 µ*M* H_2O_2 in the presence or absence of 50 µ*M* ryanodine; mRNA levels were analyzed by semiquantitative RT-PCR as previously described (23), except that β–actin was used as an internal control. (**A**) Results from a representative experiment illustrate the H_2O_2-induced increase in c-fos and egr-1 expression; this increase was not observed in cells pre-incubated with ryanodine. (**B**) The *bar graph* illustrates the mean ± SE values of PCR products, normalized to the control values determined in the absence of ryanodine. ***$p < 0.001$, ANOVA, followed by Bonferroni's posttest.

shown), generates ryanodine-sensitive calcium signals indicating that RyR calcium release channels mediate this response. Our recent studies (89) on the effects of iron on the ERK pathway and on calcium signal generation in neuronal PC12 cells have revealed that increasing cellular iron to 20 µ*M* activates the ERK pathway, while the iron chelator desferrioxamine (DFO), mannitol (an hydroxyl radical trapping agent) or ryanodine 20 µ*M* suppressed these effects (Fig. 4B, 89). These findings suggest that increasing iron promotes significant hydroxyl radical generation, which elicits RyR-mediated calcium signals that activate the ERK pathway.

Iron is a necessary element in LTP

In human infants, anemia produced by iron deficiency has been associated with altered cognitive, motor, and social-emotional outcomes. Late fetal and early postnatal iron deficiency is a common condition that causes learning and memory impairments while individuals are iron deficient, and which persist following iron repletion (13, 36, 42). Rodent models of fetal iron deficiency display significant structural and biochemical abnormalities in the hippocampus, which may predispose hippocampal area CA1 to abnormal electrophysiological responses (57). Rat pups made iron deficient during the fetal and early postnatal period show no differences in basal synaptic transmission at CA1 between iron-sufficient and iron-deficient pups at postnatal days P15 or P30. Nevertheless, the iron deficiency group does not demonstrate by P65 the expected developmental increase in synaptic strength; likewise, paired-pulse facilitation (PPF) ratios from iron-deficient slices do not exhibit the distinctive developmental changes of the iron-sufficient group (57). Hippocampal slices from iron-deficient animals show deficits in LTP even after iron repletion, since they retain a developmentally immature P15 pattern of LTP at P30, and a lower LTP pattern at P65 (57). Conversely, hippocampal brain slices prepared between postnatal day 25 and 37, obtained from rats placed on iron-deficient or control diets on gestational day 11, are not impaired in short-term PPF or long-term measurements of LTP in either the dentate gyrus (DG) or CA1 areas (82). Additionally, rats subjected to perinatal nutritional iron deficiency show impaired hippocampus-dependent learning when exposed to a fear-conditioning protocol (81). These combined results indicate that iron plays a central role in the functional development of the nervous system, and suggest that distinct hippocampal regions (*i.e.*, the DG or CA1) may be differentially compromised by developmental iron deficiency.

To explore the possible role of iron in LTP, we incubated hippocampal slices obtained from late fetal and early postnatal iron sufficient rats with the iron chelator DFO. We found that pre-incubation of slices with DFO inhibited LTP induced by tetanic stimulation (Fig. 5B and C) in hippocampal area CA1, but did not modify basal synaptic transmission or paired-pulse facilitation (Fig. 5D). These findings, which suggest strongly that iron is needed for LTP, provide new functional corroboration to the previously reported structural and biochemical abnormalities of the iron-deficient rat hippocampus (57). They also provide a potential model to account for the learning and memory deficits exhibited by humans and animals exposed to fetal or early postnatal iron deficiency, and for the persistent neurochemical and behavioral abnormalities exhibited by adult rats subjected to perinatal iron deficiency anemia, despite early iron supplementation.

CROSS TALK BETWEEN ROS/RNS, INCLUDING IRON-GENERATED ROS, AND CALCIUM IN SYNAPTIC PLASTICITY

The preceding sections have detailed how the strong activation of the hippocampal NMDA receptor during LTP-induction produces a postsynaptic increase in calcium, NO, and ROS, and how a decrease in cellular iron impairs LTP induction while an increase generates ryanodine-sensitive calcium signals and enhances ERK phosphorylation. We will discuss here how the combined increase in calcium, iron,

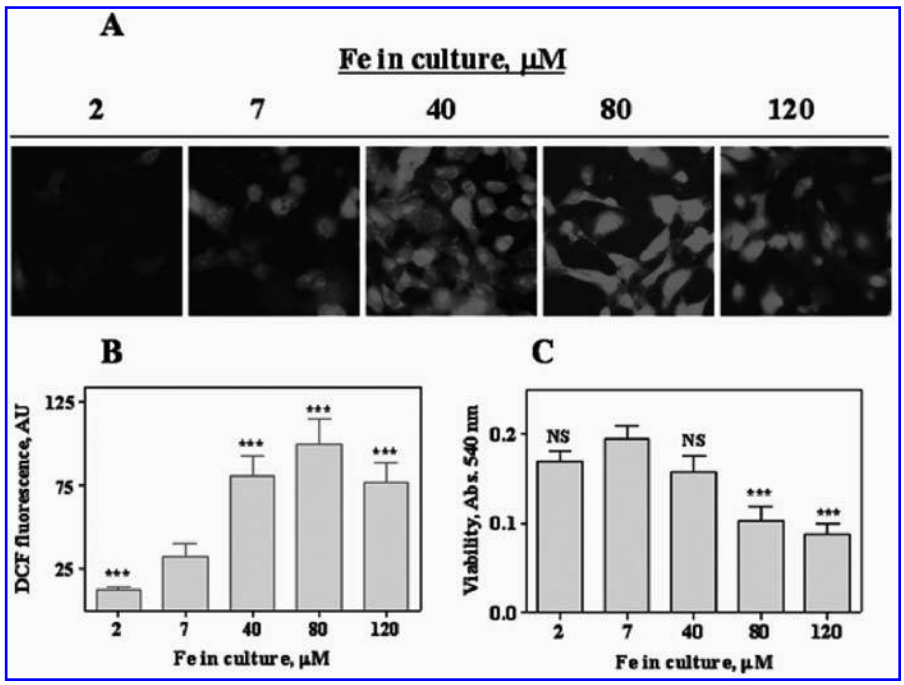

FIG. 3. Effects of increasing cellular iron content on ROS generation (A, B), and viability (C) of SH-SY5Y cells. Cells (SH-SY5Y) were grown on glass coverslips for 8 days in the standard 7 μ*M* iron medium, after which they were challenged for 2 days with medium containing 2, 7, 40, 80, or 120 μ*M* Fe. (**A**) The levels of ROS were determined by dichlorofluorescein (DCF) fluorescence, which is generated by oxidation of the nonfluorescent probe 2′, 7′-dichlorodihydrofluorescein to DCF (100). (**B**) Cell fluorescence from ten frames for each of the conditions shown in **A** was quantified using the Quantity One program (BioRad). Data are shown as mean ± SD. Cellular ROS increased when iron concentration in the culture increased from 2 to 80 μ*M*, but stabilization in ROS production was evident in the 80–120 μ*M* range. (**C**) Cells grown under similar conditions were tested for cell viability by the MTT method (87). Close to 50% of the cells died after 2 days in culture with 80 or 120 μ*M* iron (adapted from Ref. 90). ***Significantly different ($p < 0.001$); NS, not significantly different when compared to the controls grown in 7 μ*M* Fe (two-tailed ANOVA).

ROS, and NO produced by NMDA receptor activation may jointly stimulate postsynaptic RyR-mediated calcium release, producing the calcium signals required for LTP induction and maintenance. We will also discuss how changing cellular iron levels may affect RyR-mediated calcium release and synaptic plasticity. The particular focus on RyR-mediated calcium release is based on several observations, detailed below, which implicate RyR on synaptic plasticity and learning.

A requirement for RyR-mediated calcium release to elicit NMDA receptor-mediated calcium signals in hippocampal postsynaptic dendritic spines has been described (33), albeit opposing results have also been reported (68). Supporting a role for RyR in the generation of NMDA receptor-dependent calcium signals, we have found that preincubation of hippocampal neurons in culture with 50 μ*M* ryanodine blocks both the intracellular calcium increase and the stimulation of ERK phosphorylation induced by NMDA (89). Furthermore, RyR inhibition with 10 μ*M* ryanodine significantly reduces late LTP induction and activity-dependent CREB phosphorylation in postsynaptic neurons, while a lower (RyR-activating) concentration of ryanodine shifts early LTP to late LTP (76). Other experimental approaches also support a role for RyR in synaptic plasticity. In hippocampal neurons, RyR activation enhances activity-dependent release of BDNF (11) and elicits a significant increase in spine surface area (67), while treatment (3–6 h) of cultured hippocampal neurons with BDNF induces mRNA that encode for RyR2 among other synapse-associated proteins (103). Additionally, the hippocampus of rats trained in an intensive water maze task displays increased RyR2 expression, suggesting that RyR-mediated calcium release signals may be involved in memory processing after spatial learning (121).

Evidence gathered from many studies suggests strongly that cellular redox state determines RyR-mediated CICR (51). The RyR molecule contains "highly reactive" cysteine residues (defined as such by their ability to react at physiological pH), which are susceptible to modification by oxidation, *S*-nitrosylation, *S*-glutathionylation, and alkylation. By modifying these cysteine residues, H_2O_2, nitrosoglutathione, glutathione disulfide, and NO or NO donors significantly enhance RyR activity (51), while reducing agents have the opposite effects (6, 35, 37, 98). In particular, highly reduced single RyR channels from neurons barely respond *in vitro* to activation by calcium (79), even in the presence of ATP (20). Resting neurons have cytoplasmic GSH/GSSG ratios ≥ 60 (91); the resulting highly reducing potential of the neuronal cytoplasm (105) should keep RyR in a reduced state, a condition that may prevent efficient RyR activation by calcium.

The precise molecular mechanisms connecting synaptic RyR with NO-induced LTP or LTD have not been established. Based on the evidence discussed so far, we propose that RyR modification by ROS/NO generated by strong stimulation of NMDA receptors allows efficient RyR activation by the concomitant calcium entry signals. Alternatively, a role for cADPR (generated via stimulation of ADP-ribosylcyclase by NO) has been proposed (76). Thus, in neurons, cADPR may facilitate the activation of RyR-mediated calcium release by cytoplasmic calcium, as it does in sea urchin eggs (70, 71,

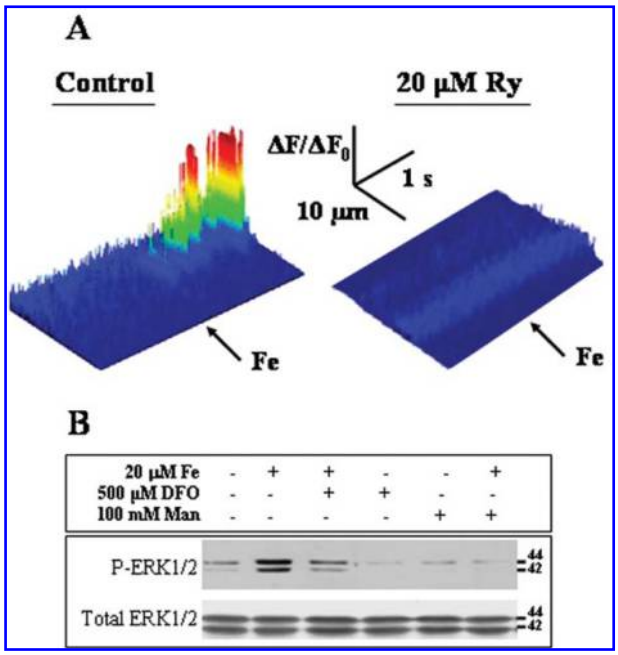

FIG. 4. Effects of iron on ryanodine-sensitive Ca^{2+} release (A) and ERK phosphorylation (B) in PC12 cells. (**A**) Control PC12 cells (*left*) or cells pretreated with 20 µ*M* ryanodine for 1 h (*right*) were loaded with the Ca^{2+} indicator Fluo 3-AM and then challenged with Fe-NTA (1: 2.2, mol: mol). Confocal line-scan images were taken at a high temporal resolution (2 ms per line) in a LSM 510 confocal microscope equipped with a CO_2- and temperature-controlled chamber. *Arrows* indicate addition of 20 µ*M* Fe-NTA. (Figure from Ref. 89, with permission from the publisher). (**B**) Quantitative Western blot analysis of ERK phosphorylation (*upper sections*) in PC12 cells exposed to 20 µ*M* Fe-NTA during 1 h or to 20 µ*M* Fe in the presence of 500 µ*M* DFO, added as iron chelator, or of 100 m*M* mannitol, added as hydroxyl radical trapper. After analysis with an antibody against phosphorylated ERKs (P-ERK1/2), the membranes were stripped and reblotted with an antibody against total ERK (Total ERK1/2). 44 and P2 indicate the migration of the 44 KDa and 42 KDa ERK subunits, respectively.

97). In either case, the resulting postsynaptic calcium signal amplification, if sufficiently large, would trigger the expression of genes involved in long-term synaptic plasticity.

The ryanodine-sensitive calcium signals induced by increasing cellular iron are blocked by the iron chelator DFO and by the hydroxyl radical trapper mannitol. These results suggest that hydroxyl radicals generated via the Fenton reaction can activate RyR-mediated calcium release (89), as shown in an earlier study of cardiac RyR (5). The mechanisms underlying RyR activation by hydroxyl radicals are unknown. The hydroxyl radical is an extremely reactive species that diffuses only a few Aº before reacting (109). If RyR had specific iron binding site(s) in which iron remained redox-active, hydroxyl radicals produced *in situ* could stimulate RyR activity by modifying amino acids present in the immediate vicinity of these putative iron binding sites. This type of mechanism was recently reported for the activation of PerR (72), a peroxide-sensing transcription factor that regulates inducible peroxide-defense genes (87). Redox-active Fe^{2+}, coordinated to residues H35, D85, H91, H93, and D104 of the PerR protein, reacts with ambient H_2O_2 via the Fenton reaction; the hydroxyl radical produced reacts with H37 or H91 causing PerR activation. The search for RyR iron binding sites may elucidate if a similar mechanism is responsible for RyR activation by hydroxyl radicals. Alternatively, iron could chemically modify RyR and stimulate its activity through more circuitous routes, such as increased NO production (40, 115) or lipid peroxidation (73, 117).

CONCLUSIONS AND PHYSIOLOGICAL IMPLICATIONS

In summary, we propose that RyR-mediated cross talk between calcium signaling and redox signaling pathways may be one of the earliest events of the postsynaptic signaling initiated by NMDA receptor activation, which culminates in long-lasting hippocampal LTP. Conditions that promote oxidative stress, such as increased iron content (119) or aging (31), may imbalance this cross communication, resulting in excessive stimulation of calcium release that, if not controlled, could induce pathological conditions or even neuronal death (95). Noteworthy, inhibition of RyR-mediated calcium release in hippocampal CA1 neurons reduces or eliminates the age-induced differences in calcium-dependent biomarkers (41). These results suggest that excessive ROS production in aging neurons (31) may cause faulty calcium homeostasis through over stimulation of RyR-mediated CICR.

ACKNOWLEDGMENTS

We thank Nancy Leal for her skillful assistance. This work was supported by FONDAP Center for Molecular Studies of the Cell, Fondo Nacional de Investigación Científica y Tecnológica (FONDECYT) Grant 15010006, by FONDECYT Grant 1060177, by Programa de Mejoramiento de la Calidad y la Equidad de la Educación Superior (MECESUP) fellowship Grant UCH001 to PM, and by project P99–031 of the Millennium Scientific Initiative, Santiago, Chile.

ABBREVIATIONS

BDNF, brain-derived nerve factor; cADPR, cyclic ADP-ribose; CICR, calcium-induced calcium release; CREB, cAMP/calcium response element binding protein; DCF, dichlorofluorescein; DFO, desferrioxamine; DG, dentate gyrus; ERK, extracellular signal-regulated kinase; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; IRE, iron responsive element; IRP, iron regulatory protein; LTD, long-term depression; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; NOX, NADPH oxidase; PPF, paired-pulse facilitation; RNS, reactive nitrogen species; ROS, reactive oxygen species; RyR, ryanodine receptor; SRF, serum response factor; TBS, theta burst stimulation.

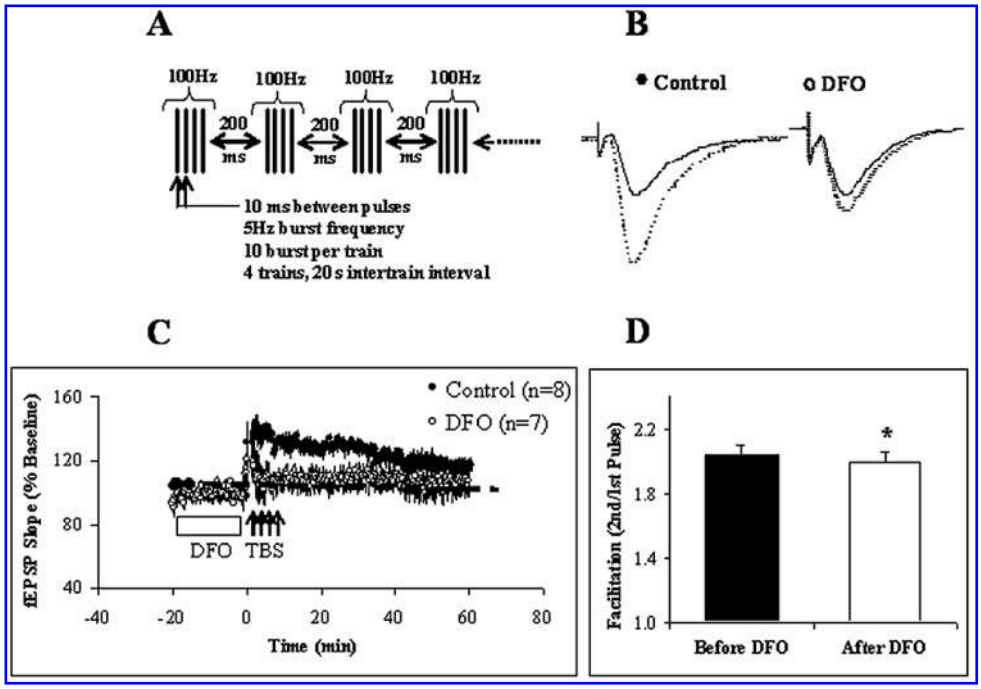

FIG. 5. Effect of the iron chelator DFO on the induction of LTP in hippocampal area CA1 by high frequency stimulation of afferent CA3 area fibers. The hippocampus from male Sprague Dawley rats (100–150 μg) was removed and 400 μm slices were prepared. The slices were perfused for 1–2 h with a standard saline solution (in m*M*: 124 NaCl, 4.4 KCl, 26 $NaHCO_3$, 10 D-glucose, 2 $CaCl_2$, and 2 $MgCl_2$, gassed with 95% O_2/5% CO_2, pH 7.4) in a chamber at 30–32°C. Baseline responses were obtained by stimulating at 0.033 Hz using an intensity that yielded half-maximal potential slopes. Test stimuli (50 μs) were given at a current (30–50 μA) that produced 50% of the maximum initial slope of the extracellular field excitatory post synaptic potential (fEPSP), which was recorded extracellularly in the CA1 stratum radiatum after stimulation with concentric bipolar electrodes. Responses to Schaffer collateral stimulation in area CA1 were monitored for a minimum of 20 min before induction of LTP. In most cases, two slices were recorded simultaneously. (**A**) Scheme of the LTP induction protocol. Four episodes of theta burst stimulation (TBS) were delivered at 0.1 Hz, using the same stimulation intensity as for baseline. For TBS, 10 stimulus trains, each composed of four pulses at 100 Hz, were delivered at 5 Hz. (**B**) Overlay of representative field potential (FP) traces, obtained in control conditions or after previous incubation of slices with 1 m*M* DFO for 20 min. One trace was taken during baseline and the other 60 min after delivery of the final train of repeated high-frequency stimulation (HFS). (**C**) Effect of DFO on LTP induced by 4x TBS. Slices were incubated for 20 min with 1 m*M* DFO; after DFO removal, basal stimulation was started and kept for 20 min before delivery of TBS; $p < 0.05$. (**D**) Comparison of paired-pulse facilitation (PPF, 40 ms interpulse interval) in control and DFO-treated groups. Data of the facilitation of the second response, given as the ratio (mean ± SE) between the slopes of the second and first response, were obtained in each condition from at least seven slices from 3 mice. * Significantly different (two-tailed p value = 0.0410) compared to the value obtained in the absence of DFO.

REFERENCES

1. Adachi T, Pimentel DR, Heibeck T, Hou X, Lee Y, Jiang B, Ido Y, and Cohen RA. *S*-glutathiolation of Ras mediates redox-sensitive signaling by angiotensin II in vascular smooth muscle cells. *J Biol Chem* 279: 29857–29862, 2004.
2. Adams JP and Sweatt JD. Molecular psychology: roles for the ERK MAP kinase cascade in memory. *Ann Rev Pharmacol Toxicol* 42: 135–163, 2002.
3. Agarwal KN. Iron and the brain: neurotransmitter receptors and magnetic resonance spectroscopy. *Br J Nutr* 85: S147–S150, 2001.
4. Aguirre P, Mena N, Tapia V, Rojas A, Arredondo M, and Núñez MT. Antioxidant responses of cortex neurons to iron loading. *Biol Res* 39: 103–104, 2006.
5. Anzai K, Ogawa K, Kuniyasu A, Ozawa T, Yamamoto H, and Nakayama H. Effects of hydroxyl radical and sulghydryl reagents on the open probability of the purified cardiac ryanodine receptor channel incorporated into planar lipid bilayers. *Biochem Biophys Res Comm* 249: 938–942, 1998.
6. Aracena P, Sánchez G, Donoso P, Hamilton SL, and Hidalgo C. *S*-glutathionylation decreases Mg^{2+} inhibition and *S*-nitrosylation enhances Ca^{2+} activation of RyR1 channels. *J Biol Chem* 278: 42927–42935, 2003.
7. Auerbach JM and Segal M. Peroxide modulation of slow onset potentiation in rat hippocampus. *J Neurosci* 17: 8695–8701, 1997.
8. Avshalumov MV, Chen BT, and Rice ME. Mechanisms underlying H(2)O(2)-mediated inhibition of synaptic transmission in rat hippocampal slices. *Brain Res* 882: 86–94, 2000.
9. Baba A, Yasui T, Fujisawa S, Yamada RX, Yamada MK, Nishiyama N, Matsuki N, and Ikegaya Y. Activity-evoked capacitative Ca^{2+} entry: Implications in synaptic plasticity. *J Neurosci* 23: 7737–7741, 2003.
10. Bailey CH, Kandel ER, and Si K. The persistence of long-term memory: a molecular approach to self-sustaining changes in learning-induced synaptic growth. *Neuron* 44: 49–57, 2004.
11. Balkowiec A and Katz DM. Cellular mechanisms regulating activity-dependent release of native brain-derived neurotrophic factor from hippocampal neurons. *J Neurosci* 22: 10399–10407, 2002.

12. Bardo S, Cavazzini MG, and Emptage N. The role of the endoplasmic reticulum Ca^{2+} store in the plasticity of central neurons. *Trends Pharmacol Sci* 27: 78–84, 2006.
13. Beard JL and Connor JR. Iron status and neural functioning. *Ann Rev Nutr* 23: 41–58, 2003.
14. Bedogni B, Pani G, Colavitti R, Riccio A, Borrello S, Murphy M, Smith R, Eboli ML, and Galeotti T. Redox regulation of CREB and induction of manganese superoxide dismutase in NGF-dependent cell survival. *J Biol Chem* 278: 16510–19519, 2003.
15. Berridge MJ. Neuronal calcium signalling. *Neuron* 21: 13–26, 1998.
16. Berridge MJ, Bootman MD, and Roderick HL Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol* 4: 517–529, 2003.
17. Bindokas VP, Jordan J, Lee CC, and Miller RJ. Superoxide production in rat hippocampal neurons: selective imaging with hydroethidine. *J Neurosci* 16: 1324–1336, 1996.
18. Bootman MD, Lipp P, and Berridge MJ. The organisation and functions of local Ca^{2+} signals. *J Cell Sci* 114: 2213–2222, 2002.
19. Boraso A and Williams AJ. Modification of the gating of the cardiac sarcoplasmic reticulum Ca^{2+}-release channel by H2O2 and dithiothreitol. *Am J Physiol* 267: H1010–H1016, 1994.
20. Bull R, Marengo JJ, Finkelstein JP, Behrens MI, and Alvarez O. SH oxidation coordinates subunits of rat brain ryanodine receptor channels activated by calcium and ATP. *Am J Physiol* 285: C119–C128, 2003.
21. Carlezon WA, Jr, Duman RS, and Nestler EJ. The many faces of CREB. *Trends Neurosci* 28: 436–445, 2005.
22. Carrasco MA, Jaimovich E, Kemmerling U, and Hidalgo C. Signal transduction and gene expression regulated by calcium release from internal stores in excitable cells. *Biol Res* 37: 701–712, 2004.
23. Carrasco MA, Riveros N, Rios J, Müller M., Torres F, Pineda J, Lantadilla S, and Jaimovich E. Depolarization induced slow transients activates early genes in skeletal muscle cells. *Am J Physiol* 284: C1438–C1447, 2003.
24. Chetkovich DM, Klann E, and Sweatt JD. Nitric oxide synthase-independent long-term potentiation in area CA1 of hippocampus. *Neuroreport* 4: 919–922, 1993.
25. Clarke DD and Sokoloff L. Circulation and energy metabolism of the brain. In: *Basic Neurochemistry: Molecular, Cellular and Medical Aspects,* edited by Siegel GJ, Agranoff BW, Albers RW, Fisher SK, and Uhler MD. New York: Lippincott-Raven, 1999, pp.637–669.
26. Colton CA and Gilbert DL. Reactive oxygen species and neuronal function. In: *Reactive Oxygen Species in Biological Systems*, edited by Gilbert CA and Colton DL. New York, NY: Kluwer Academic/Plenum Publishers, 1999, pp. 569–589.
27. Crossthwaite AJ, Hasan S, and Williams RJ. Hydrogen peroxide-mediated phosphorylation of ERK 1/2, Akt/PKB and JNK in cortical neurons: dependence on Ca^{2+} and PI3-kinase. *J Neurochem* 80: 24–35, 2002.
28. Dolmetsch R. Excitation-transcription coupling: signaling by ion channels to the nucleus. *Sci STKE* 166: PE4, 2003.
29. Dringen R, Pawlowski PG, and Hirrlinger J. Peroxide detoxification by brain cells. *J Neurosci Res* 79: 157–165, 2005.
30. Dröge W. Free radicals in the physiological control of cell function. *Physiol Rev* 82: 47–95, 2002.
31. Dröge W. Oxidative stress and ageing: is ageing a cysteine deficiency syndrome? *Phil Trans R Soc B* 360: 2355–2372, 2005.
32. Eden AN. Iron deficiency and impaired cognition in toddlers: an underestimated and undertreated problem. *Paediatr Drugs* 7: 347–352, 2005.
33. Emptage NJ, Bliss TV, and Fine A. Single synaptic events evoke NMDA receptor-mediated release of calcium from internal stores in hippocampal dendritic spines. *Neuron* 22: 115–124, 1999.
34. Emptage NJ, Reid CA, and Fine A. Calcium stores in hippocampal synaptic boutons mediate short-term plasticity, store-operated Ca^{2+} entry, and spontaneous transmitter release. *Neuron* 29: 197–208, 2001.
35. Eu JP, Sun J, Xu L, Stamler JS, and Meissner G. The skeletal muscle calcium release channel: coupled O2 sensor and NO signaling functions. *Cell* 102: 499–509, 2000.
36. Felt BT, Beard JL, Schallert T, Shao J, Aldridge JW, Connor JR, Georgieff MK, and Lozoff B. Persistent neurochemical and behavioral abnormalities in adulthood despite early iron supplementation for perinatal iron deficiency anemia in rats. *Behav Brain Res* 171: 261–270, 2006.
37. Feng W and Pessah IN. Detection of redox sensor of ryanodine receptor complexes. *Meth Enzymol* 353: 240–253, 2002.
38. Fleishmann A, Hvaldy O, Jensen T, Zacher C, Layer LE, Kvello A, Reschke M, Spanagel R, Sprengel R, Wagner EF, and Gass P. Impaired long-term memory and NR2A-type NMDA receptor-dependent synaptic plasticity in mice lacking c-fos in the CNS. *J Neurosci* 23: 9116–9122, 2003.
39. Galione A and Churchill GC. Interactions between calcium release pathways: multiple messengers and multiple stores. *Cell Calcium* 32: 343–3354, 2002.
40. Galleano M, Simontacchi M, and Puntarulo S. Nitric oxide and iron: effect of iron overload on nitric oxide production in endotoxemia. *Mol Aspects Med* 25: 141–154, 2004.
41. Gant JC, Sama MM, Landfield PW, and Thibault O. Early and simultaneous emergence of multiple hippocampal biomarkers of aging is mediated by Ca^{2+}-induced Ca^{2+} release. *J Neurosci* 26: 3482–3490, 2006.
42. Grantham–McGregor S, and Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. *J Nutr* 131: 649S–668S, 2001.
43. Guse AH. Second messenger function and the structure-activity relationship of cyclic adenosine diphosphoribose (cADPR). *FEBS J* 272: 4590–4597, 2005.
44. Gutteridge JM and Halliwell B. Free radicals and antioxidants in the year 2000. A historical look to the future. *Ann NY Acad Sci* 899: 136–147, 2000.
45. Guyton ZK, Liu Y, Gorospe M, Xu Q, and Holbrook NJ. Activation of mitogen-activated protein kinase by H2O2. Role in cell survival following oxidant injury. *J Biol Chem* 270: 4138–4142, 1996.
46. Hardingham GE, Chawla S, Cruzalegui H, and Bading H. Control of recruitment and transcription-activating function of CBP determine gene regulation by NMDA receptors and L-type calcium channels. *Neuron* 22: 789–798, 1999.
47. Hauptmann N and Cadenas E. The oxygen paradox: Biochemistry of active oxygen. In: *Oxidative Stress and the Molecular Biology of Antioxidant Defenses, e*dited by Scandalios JG. New York, NY: Cold Spring Harbor Laboratory Press, 1997, pp 1–20.
48. Hawkins RD, Son H, and Arancio O. Nitric oxide as a retrograde messenger during long-term potentiation in hippocampus. *Prog Brain Res* 118: 155–172, 1998.
49. Hentze MW and Kühn LC. Molecular control of vertebrate iron metabolism: mRNA-based regulatory circuits operated by iron, nitric oxide, and oxidative stress. *Proc Natl Acad Sci USA* 93: 8175–8182, 1996.
50. Heo J and Campbell SL. Mechanism of redox-mediated guanine nucleotide exchange on redox-active Rho GTPases. *J Biol Chem* 280: 31003–31010, 2005.
51. Hidalgo C, Donoso P, Carrasco MA. The ryanodine receptors Ca^{2+} release channels: cellular redox sensors? *IUBMB Life* 57: 315–322, 2005.
52. Hidalgo C, Sánchez G, Barrientos G, and Aracena–Parks A. A transverse tubule NOX activity stimulates calcium release from isolated triads via RYR1 *S*-glutathionylation. *J Biol Chem* 281: 26473–26482, 2006.
53. Hongpaisan J, Winters CA, and Andrews SB. Calcium-dependent mitochondrial superoxide modulates nuclear CREB phosphorylation in hippocampal neurons. *Mol Cell Neurosci* 24: 1103–1115, 2003.
54. Hubert N and Hentze MW. Previously uncharacterized isoforms of divalent metal transporter (DMT)-1: implications for regulation and cellular function. *Proc Natl Acad Sci USA* 99: 12345–12350, 2002.
55. Impey S and Goodman RH. CREB signaling-timing is everything. *Sci STKE* 82: PE1, 2001.
56. Johnson C, Hill C, and Chawla S. Calcium controls gene expression via three distinct pathways that can function independently of the Ras/mitogen-activated protein kinases (ERKs) signaling cascade. *J Neurosci* 17: 6189–6202, 1997.

57. Jorgenson LA, Sun M, O'Connor M, and Georgieff MK. Fetal iron deficiency disrupts the maturation of synaptic function and efficacy in area CA1 of the developing rat hippocampus. *Hippocampus* 15:1094–1102, 2005.
58. Kahlert S, Zundorf G, and Reiser G. Glutamate-mediated influx of extracellular Ca^{2+} is coupled with reactive oxygen species generation in cultured hippocampal neurons but not in astrocytes. *J Neurosci Res* 79: 262–271, 2005.
59. Kamsler A and Segal M. Paradoxical actions of hydrogen peroxide on long-term potentiation in transgenic superoxide dismutase-1 mice. *J Neurosci* 23: 269–276, 2003.
60. Kamsler A and Segal M. Hydrogen peroxide as a diffusible signal molecule in synaptic plasticity. *Mol Neurobiol* 29: 167–178, 2004.
61. Kanterewicz BI, Knapp LT, and Klann E. Stimulation of p42 and p44 mitogen-activated protein kinases by reactive oxygen species and nitric oxide in hippocampus. *J Neurochem* 70: 1009–1016, 1998.
62. Katsuki H, Nakanishi C, Saito H, and Matsuki N. Biphasic effect of hydrogen peroxide on field potentials in rat hippocampal slices. *Eur J Pharmacol* 337: 213–218, 1997.
63. Kelleher III RJ, Govindarajan A, Jung H-Y, Kang H, and Tonegawa S. Translational control by MAPK signaling in long-term synaptic plasticity and memory. *Cell* 116: 467–479, 2004.
64. Kemmerling U, Munoz P, Müller M, Sánchez G, Aylwin ML, Klann E, Carrasco MA, and Hidalgo C. Calcium release by ryanodine receptors mediates hydrogen peroxide-induced activation of ERK and CREB phosphorylation in N2a cells and hippocampal neurons. *Cell Calcium* (2006), doi:10:1016/j.ceca.2006.10.001.
65. Kishida KT, Pao M, Holland SM, and Klann E. NADPH oxidase is required for NMDA receptor-dependent activation of ERK in hippocampal area CA1. *J Neurochem* 94: 299–306, 2005.
66. Klann E. Cell-permeable scavengers of superoxide prevent long-term potentiation in hippocampal area CA1. *J Neurophysiol* 80: 452–457, 1998.
67. Korkotian E and Segal M. Release of calcium stores alters the morphology of dendritic spines in cultured hippocampal neurons. *Proc Nat Acad Sci USA* 96: 12068–12072, 1999.
68. Kovalchuk Y, Eilers J, Lisman J, and Konnerth A. NMDA receptor-mediated subthreshold Ca(2+) signals in spines of hippocampal neurons. *J Neurosci* 20: 1791–1799, 2000.
69. Krapivinsky G, Krapivinsky L, Manasian Y, Ivanov A, Tyzio R, Pellegrino C, Ben–Ari Y, Clapham D, and Medina I. The NMDA receptor is coupled to the ERK pathway by a direct interaction between NR2B and RasGRF1. *Neuron* 40: 775–784, 2003.
70. Lee HC. Potentiation of calcium- and caffeine-induced calcium release by cyclic ADP-ribose. *J Biol Chem* 268: 293–299, 1993
71. Lee HC, Aarhus R, and Graeff RM. Sensitization of calcium-induced calcium release by cyclic ADP-ribose and calmodulin. *J Biol Chem* 270: 9060–9066, 1995.
72. Lee JW and Helmann JD. The PerR transcription factor senses H2O2 by metal-catalysed histidine oxidation. *Nature* 440: 363–367, 2006.
73. Lee SH, Oe T, Arora JS, and Blair IA. Analysis of FeII-mediated decomposition of a linoleic acid-derived lipid hydroperoxide by liquid chromatography/mass spectrometry. *J Mass Spectrom* 40:661–668, 2005.
74. Lin HH, Chen CH, Hsieh WK, and Chiu TH. Hydrogen peroxide increases the activity of rat sympathetic preganglionic neurons *in vivo* and *in vitro*. *Neuroscience* 121: 641–647, 2003.
75. Lozoff B, Jimenez E, and Wolf AW. Long-term developmental outcome of infants with iron deficiency. *N Engl J Med* 325: 687–694, 1991.
76. Lu YF and Hawkins RD. Ryanodine receptors contribute to cGMP-induced late-phase LTP and CREB phosphorylation in the hippocampus. *J Neurophysiol* 88: 1270–1278, 2002.
77. Lynch MA. Long-term potentiation and memory. *Physiol Rev* 84: 87–136, 2004.
78. Malenka RC and Bear MF. LTP and LTD: an embarrassment of riches. *Neuron* 44: 5–21, 2004.
79. Marengo JJ, Hidalgo C, and Bull R. Sulfhydryl oxidation modifies the calcium dependence of ryanodine-sensitive calcium channels of excitable cells. *Biophys J* 74: 1263–1277, 1998.
80. Marie H, Morishita W, Yu X, Calakos N, and Malenka RC. Generation of silent synapses by acute *in vivo* expression of CaMKIV and CREB. *Neuron* 45: 741–752, 2005.
81. McEchron MD, Cheng AY, Liu H, Connor JR, and Gilmartin MR. Perinatal nutritional iron deficiency permanently impairs hippocampus-dependent trace fear conditioning in rats. *Nutr Neurosci* 8: 195–206, 2005.
82. McEchron MD and Paronish MD. Perinatal nutritional iron deficiency reduces hippocampal synaptic transmission but does not impair short- or long-term synaptic plasticity. *Nutr Neurosci* 8: 277–285, 2005.
83. McGahan MC, Harned J, Mukunnemkeril M, Goralska M, Fleisher L, and Ferrell JB. Iron alters glutamate secretion by regulating cytosolic aconitase activity. *Am J Physiol* 288: C1117–C1124, 2005.
84. Meldolesi J. Rapidly exchanging Ca^{2+} stores in neurons: Molecular, structural and functional properties. *Progress Neurobiol* 65: 309–338, 2001.
85. Mellström B and Naranjo J.R. Mechanism of Ca^{++}-dependent transcription. *Curr Op Neurobiol* 11: 312–319, 2001.
86. Mellström B, Torres B, Link WA, and Naranjo JR The BDNF gene: exemplifying complexity in Ca^{2+} -dependent gene expression. *Crit Rev Neurobiol* 16: 43–49, 2004.
86. Mongkolsuk S and Helmann JD. Regulation of inducible peroxide stress responses. *Mol Microbiol* 45: 9–15, 2002.
87. Mossman T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Meth* 65: 55–63; 1986.
89. Muñoz P, Zavala G, Castillo K, Aguirre P, Hidalgo C and Núñez MT. Effect of iron on the activation of the MAPK/ERK pathway in PC12 neuroblastoma cells. *Biol Res* 39: 189–190, 2006.
90. Mura CV, Delgado R, Aguirre P, Bacigalupo J, and Núñez MT. Quiescence induced by iron challenge protects neuroblastoma cells from oxidative stress. *J Neurochem* 98: 11–19, 2006.
91. Núñez MT, Gallardo V, Muñoz P, Tapia V, Esparza A, Salazar J, and Speisky H. Progressive iron accumulation induces a biphasic change in the glutathione content of neuroblastoma cells. *Free Radic Biol Med* 37: 953–960, 2004.
92. Núñez MT, Núñez–Millacura C, Tapia V, Muñoz P, Mazariegos D, Arredondo M, Muñoz P, Mura C, and Maccioni RB. Iron-activated iron uptake: a positive feedback loop mediated by iron regulatory protein 1. *Biometals* 16: 83–90, 2003.
93. Núñez–Millacura C, Tapia V, Muñoz P, Maccioni RB, and Núñez MT. An oxidative stress-mediated positive-feedback iron uptake loop in neuronal cells. *J Neurochem* 82: 240–248, 2002.
94. Oba T, Kurono C, Nakajima R, Takaishi T, Ishida K, Fuller GA, Klomkeaw W and Yamaguchi M. H2O2 activates ryanodine receptor but has little effect on recovery of releasable Ca^{2+} content after fatigue. *J Appl Physiol* 93: 1999–2008, 2002.
95. Orrenius S, Zhivotsky B, and Nicotera P. Regulation of cell death: the calcium-apoptosis link. *Nature Rev Mol Cell Biol* 4: 552–565, 2003.
96. Ortiz E, Pasquini JM, Thompson K, Felt B, Butkus G, Beard J, and Connor JR. Effect of manipulation of iron storage, transport, or availability on myelin composition and brain iron content in three different animal models. *J Neurosci Res* 77: 681–689, 2004.
97. Perez CF, Marengo JJ, Bull R and Hidalgo C. Cyclic ADP-ribose activates caffeine-sensitive calcium channels from sea urchin egg microsomes. *Am J Physiol* 274: C430–C439, 1998.
98. Pessah IN, Kim KH, and Feng W. Redox sensing properties of the ryanodine receptor complex. *Frontiers in Bioscience* 7: 72–79, 2002.
99. Peunova N and Enikolopov G. Amplification of calcium-induced gene transcription by nitric oxide in neuronal cells. *Nature* 364: 450–453, 1993.
100. Reyes–Harde M, Potter BVL, Galione A, and Stanton PK. Induction of hippocampal LTD requires nitric-oxide-stimulated PKG activity and Ca^{2+} release from cyclic ADP-ribose-sensitive stores. *J Neurophysiol* 82: 1569–1576, 1999.
101. Reynolds IJ and Hastings T. Glutamate induces the production of reactive oxygen species in cultured forebrain neurons following NMDA receptor activation. *J Neurosci* 15: 3318–3327, 1995.

102. Richards DA, Bliss TV, and Richards CD. Differential modulation of NMDA-induced calcium transients by arachidonic acid and nitric oxide in cultured hippocampal neurons. *Eur J Neurosci* 17: 2323–2328, 2003.
103. Ring RH, Alder J, Fennell M, KourANOVA E, Black IB, and Thakker–Varia S. Transcriptional profiling of brain-derived neurotrophic factor-induced neuronal plasticity: a novel role for nociceptin in hippocampal neurite outgrowth. *J Neurobiol* 66: 361–377, 2006.
104. Sánchez G, Pedrozo Z, Domenech RJ, Hidalgo C, and Donoso P. Tachycardia increases NADPH oxidase activity and RyR2 S-glutathionylation in ventricular muscle. *J Mol Cell Cardiol* 39: 982–991, 2005.
104. Schafer FQ and Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic Biol Med* 30: 1191–1212, 2001.
106. Serrano F and Klann E. Reactive oxygen species and synaptic plasticity in the aging hippocampus. *Ageing Res Rev* 3: 431–443, 2004.
107. Serrano F, Kolluri NS, Wientjes FB, Card JP, and Klann E. NADPH oxidase immunoreactivity in the mouse brain. *Brain Res* 988: 193–198, 2003.
108. Svoboda K and Mainen ZF. Synaptic [Ca^{2+}]: Intracelular stores spill their guts. *Neuron* 22: 427–430, 1999.
109. Symons MCR and Gutteridge JMC (Eds). *Free Radicals and Iron: Chemistry, Biology and Medicine.* New York, NY: Oxford University Press, 1998, pp. 40–60.
110. Tejada–Simon MV, Serrano F, Villasana LE, Kanterewicz BI, Wu GY, Quinn MT, and Klann E. Synaptic localization of a functional NADPH oxidase in the mouse hippocampus. *Mol Cell Neurosci* 29: 97–106, 2005.
111. Thiels E, Urban NN, González–Burgos GR, Kanterewicz BI, Barrionuevo G, Chu CT, Ourv TD, and Klann E. Impairment of long-term potentiation and associative memory in mice that overexpress extracellular superoxide dismutase. *J Neurosci* 20: 7631–7639, 2000.
112. Thomas GM and Huganir RL. MAPK cascade signalling and synaptic plasticity. *Nat Rev Neurosci* 5: 173–183, 2004.
113. West AE, Griffith EC, and Greenberg ME. Regulation of transcription factors by neuronal activity. *Nature Rev Neurosci* 3: 921–931, 2002.
114. Wu G, Deisseroth K, and Tsien RW. Activity-dependent CREB phosphorylation: Convergence of a fast, sensitive calmoduline kinase pathway and a slow, less sensitive mitogen-activated protein kinase pathway. *Proc Natl Acad Sci USA* 98: 2808–2813, 2001.
115. Xu M, Wai–Cheong YG, Gan LT, and Ng YK. Distinct roles of oxidative stress and antioxidants in the nucleus dorsalis and red nucleus following spinal cord hemisection. *Brain Res* 1055: 137–142, 2005.
116. Yermolaieva O, Brot N, Weissbach H, Heinemann SH, and Hoshi T. Reactive oxygen species and nitric oxide mediate plasticity of neuronal calcium signalling. *Proc Natl Acad Sci USA* 97: 448–453, 2000.
117. Yoshida K, Kaneko K, Miyajima H, Tokuda T, Nakamura A, Kato M, and Ikeda S. Increased lipid peroxidation in the brains of aceruloplasminemia patients. *J Neurol Sci* 175: 91–95, 2000.
118. Yoshizumi M, Kogame T, Suzaki Y, Fujita Y, Kyaw M, Kirima K, Ishizawa K, Tsuchiya K, Kagami S, and Tamaki T. Ebselen attenuates oxidative stress-induced apoptosis via the inhibition of the c-jun N-terminal kinase and activator protein-1 signalling pathway in PC12 cells. *Br J Pharmacol* 136: 1023–1032, 2002.
119. Zecca L, Gallorini M, Schunemann V, Trautwein AX, Gerlach M, Riederer P, Vezzoni P, and Tampellini D. Iron, neuromelanin and ferritin content in the substantia nigra of normal subjects at different ages: consequences for iron storage and neurodegenerative processes. *J Neurochem* 76: 1766–1773, 2001.
120. Zhang L and Jope R. Oxidative stress differentially modulates phosphorylation of ERK, p38 and CREB induced by NGF or EGF in PC12 cells. *Neurobiol Aging* 20: 271–278, 1999.
121. Zhao W, Meiri N, Cavallaro S, Quattrone A, Zhang L, and Alkon DL. Spatial learning induced changes in expression of the ryanodine type II receptor in the rat hippocampus. *FASEB J* 14: 290–300, 2000.

Address reprint requests to:
Professor Cecilia Hidalgo
ICBM, Facultad de Medicina
Universidad de Chile
Casilla 70005
Santiago 7, Chile

E-mail: chidalgo@med.uchile.cl

Date of first submission to ARS Central, August 27, 2006; date of acceptance, September 10, 2006.

This article has been cited by:

1. István Bókkon, Attila Till, Friedrich Grass, Attila Erdöfi Szabó. 2011. Phantom pain reduction by low-frequency and low-intensity electromagnetic fields. *Electromagnetic Biology and Medicine* **30**:3, 115-127. [CrossRef]
2. A. Tsentsevitsky, E. Nikolsky, R. Giniatullin, E. Bukharaeva. 2011. Opposite modulation of time course of quantal release in two parts of the same synapse by reactive oxygen species. *Neuroscience* **189**, 93-99. [CrossRef]
3. Cynthia A. Massaad , Eric Klann . 2011. Reactive Oxygen Species in the Regulation of Synaptic Plasticity and Memory. *Antioxidants & Redox Signaling* **14**:10, 2013-2054. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
4. Denise Riquelme , Alvaro Alvarez , Nancy Leal , Tatiana Adasme , Italo Espinoza , Juan Antonio Valdés , Natalia Troncoso , Steffen Hartel , Jorge Hidalgo , Cecilia Hidalgo , M. Angélica Carrasco . 2011. High-Frequency Field Stimulation of Primary Neurons Enhances Ryanodine Receptor-Mediated Ca2+ Release and Generates Hydrogen Peroxide, Which Jointly Stimulate NF-#B Activity. *Antioxidants & Redox Signaling* **14**:7, 1245-1259. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
5. MAJID RAHNAMA, JACK A. TUSZYNSKI, ISTVÁN BÓKKON, MICHAL CIFRA, PEYMAN SARDAR, VAHID SALARI. 2011. EMISSION OF MITOCHONDRIAL BIOPHOTONS AND THEIR EFFECT ON ELECTRICAL ACTIVITY OF MEMBRANE VIA MICROTUBULES. *Journal of Integrative Neuroscience* **10**:01, 65-88. [CrossRef]
6. Orly Weinreb , Tamar Amit , Silvia Mandel , Lana Kupershmidt , Moussa B.H. Youdim . 2010. Neuroprotective Multifunctional Iron Chelators: From Redox-Sensitive Process to Novel Therapeutic Opportunities. *Antioxidants & Redox Signaling* **13**:6, 919-949. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
7. I. Bókkon, V. Salari, J.A. Tuszynski, I. Antal. 2010. Estimation of the number of biophotons involved in the visual perception of a single-object image: Biophoton intensity can be considerably higher inside cells than outside. *Journal of Photochemistry and Photobiology B: Biology* **100**:3, 160-166. [CrossRef]
8. Andreas Wolfram Henkel, Oliver Welzel, Teja Wolfgang Groemer, Philipp Tripal, Andrea Rotter, Johannes Kornhuber. 2010. Fluoxetine prevents stimulation-dependent fatigue of synaptic vesicle exocytosis in hippocampal neurons. *Journal of Neurochemistry* **114**:3, 697-705. [CrossRef]
9. Paola Haeger, Álvaro Álvarez, Nancy Leal, Tatiana Adasme, Marco Tulio Núñez, Cecilia Hidalgo. 2010. Increased Hippocampal Expression of the Divalent Metal Transporter 1 (DMT1) mRNA Variants 1B and +IRE and DMT1 Protein After NMDA-Receptor Stimulation or Spatial Memory Training. *Neurotoxicity Research* **17**:3, 238-247. [CrossRef]
10. Silvia Sorce , Karl-Heinz Krause . 2009. NOX Enzymes in the Central Nervous System: From Signaling to Disease. *Antioxidants & Redox Signaling* **11**:10, 2481-2504. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
11. Hiroshi Kobayashi, Yoshihiko Yamada, Seiji Kanayama, Naoto Furukawa, Taketoshi Noguchi, Shoji Haruta, Shozo Yoshida, Mariko Sakata, Toshiyuki Sado, Hidekazu Oi. 2009. The Role of Hepatocyte Nuclear Factor-1# in the Pathogenesis of Clear Cell Carcinoma of the Ovary. *International Journal of Gynecological Cancer* **19**:3, 471-479. [CrossRef]
12. C. David Rollo. 2009. Dopamine and Aging: Intersecting Facets. *Neurochemical Research* **34**:4, 601-629. [CrossRef]
13. David G. Thomas, Stephanie L. Grant, Nicki L. Aubuchon-Endsley. 2009. The Role of Iron in Neurocognitive Development. *Developmental Neuropsychology* **34**:2, 196-222. [CrossRef]
14. G. Zündorf, S. Kahlert, V.I. Bunik, G. Reiser. 2009. #-Ketoglutarate dehydrogenase contributes to production of reactive oxygen species in glutamate-stimulated hippocampal neurons in situ. *Neuroscience* **158**:2, 610-616. [CrossRef]
15. E EISENBERG, S SHTAHL, R GELLER, A REZNICK, O SHARF, M RAVBINOVICH, A ERENREICH, R NAGLER. 2008. Serum and salivary oxidative analysis in Complex Regional Pain Syndrome. *Pain* **138**:1, 226-232. [CrossRef]
16. A. Minelli, I. Bellezza, S. Grottelli, F. Galli. 2008. Focus on cyclo(His-Pro): history and perspectives as antioxidant peptide. *Amino Acids* **35**:2, 283-289. [CrossRef]
17. Cecilia Hidalgo , Paulina Donoso . 2008. Crosstalk Between Calcium and Redox Signaling: From Molecular Mechanisms to Health Implications. *Antioxidants & Redox Signaling* **10**:7, 1275-1312. [Abstract] [Full Text PDF] [Full Text PDF with Links]
18. E MILLEROTSERRUROT, N BERTRAND, C MOSSIAT, P FAURE, A PRIGENTTESSIER, P GARNIER, Y BEJOT, M GIROUD, A BELEY, C MARIE. 2008. Temporal changes in free iron levels after brain ischemiaRelevance to the timing of iron chelation therapy in stroke. *Neurochemistry International* **52**:8, 1442-1448. [CrossRef]
19. Dr. Ariel Kamsler , Menahem Segal . 2007. Control of Neuronal Plasticity by Reactive Oxygen Species. *Antioxidants & Redox Signaling* **9**:2, 165-167. [Citation] [Full Text PDF] [Full Text PDF with Links]