

Iron-mediated redox modulation in neural plasticity

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The role of iron in brain physiology has focused on the neuropathological, effects due to iron-induced oxidative stress. However, our recent work has established a physiological relationship between the iron-mediated oxidative modification and normal neuronal function. Our results obtained from hippocampal neurons, suggest that iron-generated reactive species oxygen (ROS) are involved in calcium signaling initiated by stimulation of NMDA receptors. This signal is amplified by ryanodine receptors (RyR), a redox-sensitive calcium channel, allowing the phosphorylation and nuclear translocation of ERK1/2. Furthermore, using electrophysiological approaches, we showed that iron is required for basal synaptic transmission and full expression of long-term potentiation, a type of synaptic plasticity. Our data combined suggest that the oxidative effect of iron is critical to activate processes that are downstream of NMDAR activation. Finally, due to the high reactivity of DNA with iron-generated ROS, we hypothesize an additional function of iron in gene regulation.

Keywords: iron, ryanodine receptor, synaptic plasticity, redox signaling, gene expression

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Due to its ability to accept or transfer electrons, iron participates in a series of redox reactions^{1,2} such as the Fenton reaction that generates hydroxyl free radicals or the Haber-Weiss reaction that combines the reduction of Fe³⁺ by superoxide plus oxygen to produce Fe²⁺.

When iron accumulates, it can promote oxidative stress which in turn triggers neuronal death.³ As a result of iron-mediated reactions, the study of iron in the brain has been focused on its neuropathological role.^{4,5} However, our recent work has established a physiological

relationship between the iron-mediated oxidative modification and normal neuronal function.⁶ This work also suggests that iron-generated reactive species oxygen (ROS) could be a new class of molecules that act as second messengers in signaling cascades related to synaptic plasticity (SP), the putative cellular substrate of memory.

Consistent with a potential physiological role of iron, the activation of N-Methyl-D-aspartate (NMDA) receptors (NMDAR) induces iron uptake in cultured cortical neurons (Fig. 1), which in turn induces the production of the hydroxyl free radical-mediated Fenton reaction.² Similarly, in cultured hippocampal neurons, we showed that the entry of iron rapidly increased labile iron for the Fenton reaction.⁶

Why are there different mechanisms to incorporate iron, after neuronal activity? We propose that iron uptake is needed to provide a neuronal oxidative tone necessary for the proper functioning and operation of components sensitive to this tone. In agreement with this idea, ryanodine receptors (RyR), a channel involved in calcium-induced calcium release (CICR) which activates various signaling pathways involved in synaptic plasticity,⁷ has a group of oxidation-sensitive cysteines⁸ that could be one of the putative targets of iron-mediated oxidative attacks, as proposed by earlier

work in PC12 cells.⁹ Our latest work in hippocampal neurons suggests that iron-generated ROS are involved in calcium signaling initiated by stimulation of NMDA receptors and amplified by the RyR.⁶ An iron chelator, deferrioxamine, prevents CICR as well as the phosphorylation and nuclear translocation of ERK 1/2 necessary to establish synaptic plasticity (SP) and gene expression-dependent

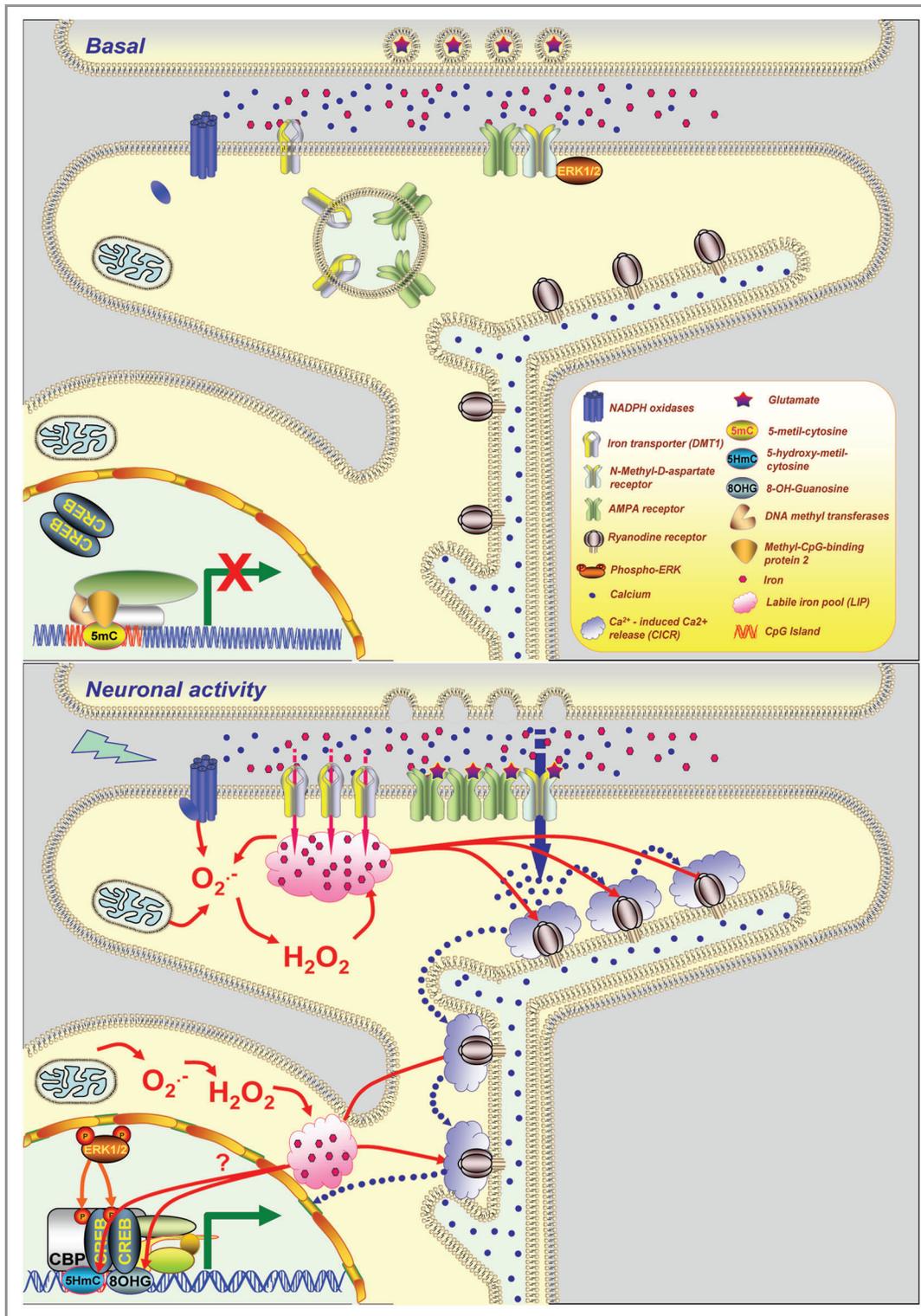


Figure 1. For figure legend, see page 168.

CREB (Fig. 1). Furthermore, using electrophysiological approaches, we showed that iron is required for basal synaptic transmission and full expression of

long-term potentiation (LTP), suggesting that ROS generated by iron is an important component for SP. Recent work showing that generation of

ROS-dependent activation of NMDAR is required for the SP and hippocampal-dependent memory¹⁰ is in agreement with the potential role of iron in SP.

Figure 1 (See previous page). Under basal condition (Fig. 1A), glutamatergic synapses in the hippocampal CA1 region is coupled to proteins required to induce synaptic plasticity (SP), such as N-Methyl-D-aspartate receptors (NMDAR) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA), divalent metal transporter 1 (DMT1, Nramp1), neuronal NADPH oxidase and a large number of kinases that are activated by calcium (e.g.: ERK 1/2). At hippocampal dendrites, the endoplasmic reticulum, an intracellular calcium reservoir containing the ryanodine receptors (RyR), which are activated by calcium and reactive oxygen species (ROS). In the nucleus, regulatory sequences within the genes involved in SP (CpG islands), are frequently methylated by DNA methyltransferases (DNMTs). Methylation of cytosine at position 5, leads to the formation of 5-methyl-cytosine (5mC) which is recognized by methyl CpG binding protein 2 (MeCP2) preventing the binding of transcription factors activated by calcium, such as cAMP response element-binding (CREB). Neuronal activity (Fig. 1B) triggers critical events for expression of SP, such as insertion of AMPAR to the synapse; superoxide generation and subsequent dismutation to hydrogen peroxide; iron uptake that promotes oxidative reactions; NMDAR activation that allows the calcium influx from the synaptic cleft, which coupled with redox modifications of RyRs, amplifies the signal of calcium in a process known as calcium-induced calcium release. This process activates calcium signaling pathway such as ERK/MAPKs that phosphorylate the transcription factor CREB, regulating genes involved in SP. Iron may modulate the binding of these transcriptional regulators, forming adducts such as 8-hydroxyguanosine (8OHG) or 5 hydroxymethylcytosine (5HmC).

There is intensive research into the identification of other molecular targets of iron-mediated oxidation, although to date not all processes modulated by iron have been uncovered.

Given the high sensibility of DNA to iron-generated ROS,¹¹ it is intriguing to speculate about the normal function of iron in gene regulatory mechanisms. In particular, methylation of cytosine residues that are adjacent to a guanosine base is an epigenetic mechanism that has been implicated in the regulation of genes involved in SP and memory.^{12,13} Interestingly, methylation sites are frequently clustered in regulatory regions called CpG islands, which are particularly

sensitive to oxidative damage.¹⁴ Thus, iron-dependent oxidative reactions could also regulate gene expression by oxidizing DNA; leading to the formation of different adduct bases which may alter gene expression (Fig. 1). In fact, the 8-hydroxyguanosine (8OHG), has been described as a marker of DNA damage.¹¹ However, the 5-hydroxymethylcytosine (5hmC) is formed by enzymatic oxidation over the 5-methylcytosine catalyzed by a family of iron-dependent dioxygenases, recently identified in neurons.^{15,16} The functional significance of these oxidative modifications is unknown; however, they have been involved in the dynamics of DNA methylation.

Finally, combined data from our study suggest that the oxidative effect of iron is critical to activate processes that are downstream of NMDAR activation which are required for SP, like calcium release from ryanodine-sensitive intracellular store or gene expression.

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