Calcium, Reactive Oxygen Species, and Synaptic Plasticity

In this review article, we address how activity-dependent $\text{Ca}^{2+}$ signaling is crucial for hippocampal synaptic/structural plasticity and discuss how changes in neuronal oxidative state affect $\text{Ca}^{2+}$ signaling and synaptic plasticity. We also analyze current evidence indicating that oxidative stress and abnormal $\text{Ca}^{2+}$ signaling contribute to age-related synaptic plasticity deterioration.

Synaptic Plasticity, Learning, and Memory

The brain is a highly plastic organ that undergoes constant changes in structure and function in response to experience. This remarkable plasticity allows the brain to acquire, store, and retrieve information via memory, a fundamental aspect of human cognition. The hippocampus is a brain region with a key role in spatial learning and declarative memory, as evidenced by the deleterious effects of hippocampal damage caused by trauma, aging, or neurodegenerative processes on these responses (19, 39, 186, 192, 193, 213). Consequently, identifying the hippocampal neuronal pathways underlying these cognitive functions is essential to understand how the brain receives, decodes, stores, and uses the stored information. Modification of the efficacy of synaptic transmission in response to neuronal activity (the classic definition of synaptic plasticity) represents a key mechanism whereby experience-induced neuronal activity modifies the function of the brain. Whereas the last decades have generated significant knowledge on the cellular and molecular mechanisms that produce synaptic plasticity in the hippocampus, in this review, we will focus on how cross talk between activity-dependent signaling mediated by $\text{Ca}^{2+}$ and reactive oxygen species (ROS) impinges on synaptic plasticity in young and aged hippocampus. Knowledge gained from studies on this emerging topic is bound to further our current understanding of the cellular basis of learning and memory.

Synaptic plasticity, which has been extensively studied in the last decades using electrophysiological methods (27), also entails structural changes in synapses that cause the structural plasticity responses exemplified by dendritic spine remodeling (13, 212). Present evidence supports these two related forms of plasticity as the biological substrates for associative learning and long-term memory processes (14, 15, 20, 26, 44, 60, 65, 68, 75, 92, 110, 112, 132, 143, 145, 151, 164, 207). As stated above, the hippocampus is essential for spatial learning and memory formation. Hence, we will concentrate on the cellular pathways underlying hippocampal synaptic/structural plasticity, focusing on the key roles played by $\text{Ca}^{2+}$ and ROS signaling, and on how changes in these signaling pathways during aging deteriorate synaptic plasticity, resulting in defective learning and memory formation.

Long-Term Potentiation and Long-Term Depression

Activity-dependent changes in synaptic transmission include long-term potentiation (LTP), manifested by an increase in synaptic efficacy, and long-term depression (LTD), which represents a decrease in synaptic efficacy caused by more prolonged low-frequency stimulation (21, 41, 147). Due to the central role played by both LTP and LTD in cognitive processes, it becomes essential to identify in detail the molecular and cellular events responsible for both neuronal responses and to decipher how these entities progressively fail during aging. Calcium signaling has a well-established and central role in both LTP and LTD, as detailed below. Although there is less information on how ROS signaling affects these responses, increasing evidence indicates that neuronal redox state, such as the increased oxidative tone displayed by neurons during aging, has a significant influence on neuronal $\text{Ca}^{2+}$ signaling. Hence, identifying the cellular and molecular entities engaged in activity-dependent cross talk between neuronal $\text{Ca}^{2+}$ and ROS signaling and unraveling their effects on LTP and LTD is an emerging subject in neuroscience worthy of consideration and further research.

As described above, a strong correlation exists between LTP and hippocampus-dependent learning and memory formation. Therefore, LTP represents at present a preferential candidate mechanism for brain information storage. In addition, increasing evidence supports a role for LTD as a genuine learning and memory mechanism in mammalian brain (45, 57, 131). A role for LTD as a homeostatic mechanism to ensure that hippocampal synapses are not saturated by learning, or in mediating learning, forgetting, or
behavioral extinction was proposed some years ago (46). Different protocols involving trains of brief bursts of tetanic stimulation induce hippocampal LTP. The two most frequently employed LTP-inducing protocols include high-frequency stimulation (HFS) delivered at 100 Hz, and theta-burst stimulation (TBS) delivered at the theta frequency of 4–7 Hz (FIGURE 1A). Of these two protocols, TBS resembles the type of oscillatory frequency recorded in rodents during exploration tasks (127). Low-frequency stimulation (FIGURE 1A) and paired-pulse low-frequency stimulation represent widespread protocols to induce LTD (41).

Here, we will focus mainly on hippocampal N-methyl-D-aspartate (NMDA) receptor-dependent LTP/LTD generated at the synapse engaging Schaffer collateral/commissural fibers projecting from cornus ammonis 3 (CA3) to cornus ammonis 1 (CA1) pyramidal neurons. At these synapses, tetanic stimulation of Schaffer collaterals induces LTP in area CA1 by increasing net Ca\(^{2+}\) entry flux via activation of postsynaptic calcium-permeable NMDA receptors (26, 43, 149, 159, 160). These receptors act as molecular coincidence detectors through the concurrent activity of pre- and postsynaptic neurons; Ca\(^{2+}\) influx through NMDA receptor channels requires glutamate and glycine or D-serine binding in addition to the depolarization produced by glutamate activation of postsynaptic \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors, which displaces Mg\(^{2+}\) from its channel-blocking site (26). Activation of postsynaptic NMDA receptors can induce either LTP or LTD (142). As described below, these responses depend on the magnitude and duration of

![Diagram of synaptic plasticity and activity-induced spine remodeling](http://physiologyonline.physiology.org/)

**FIGURE 1.** Synaptic plasticity and activity-induced spine remodeling

A: the left panels indicate the most commonly used stimulation protocols to induce LTP by high-frequency or theta-burst stimulation, and LTD by low-frequency stimulation. The middle panels show the CA3-CA1 hippocampal circuit and an example of a field excitatory postsynaptic potential (fEPSP) record, indicating its slope; changes in fEPSP slope reflect changes in hippocampal postsynaptic excitatory activity. The right panels show the prototypical LTP and LTD responses, exemplified by increased or decreased fEPSP slopes after stimulation. B: scheme showing dendritic spine remodeling in response to neuronal activity. Dendritic spines exhibit a variety of shapes and sizes and undergo significant remodeling during activity-dependent plasticity. At hippocampal synapses, sustained LTP or LTD both affect spine morphology. The dendritic spine remodeling induced by LTP entails new spine growth and enlargement of preexisting spines, whereas LTD has been associated with spine shrinkage and spine elimination.
postsynaptic Ca\(^{2+}\) signals, which represent transient increases in intracellular free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]). The phosphorylation levels (129, 130) and the redistribution (142) of AMPA receptors mediate the early phases of LTP and LTD; addition or enhanced phosphorylation of AMPA receptors result in LTP, whereas their removal or dephosphorylation weaken synaptic strength and induce LTD. With time, LTP-associated changes promote the synthesis of new proteins, giving rise to lasting structural changes.

Since the discovery more than 40 years ago of HFS-induced LTP in the perforant path/dentate gyrus synapse of rabbit hippocampus (28), numerous reports have addressed the mechanisms underlying the LTP response, mostly at glutamatergic excitatory synapses of rodent hippocampus. In 2007, Clarke R. Raymond described different phases of LTP (179). The short-lasting (30–60 min) LTP phase, also known as short-term potentiation, depends on posttranslational modifications of strategic synaptic proteins, is not affected by most protein kinase inhibitors, and does not require transcription and translation. The next LTP phase, which engages Ca\(^{2+}\)-dependent activation of protein kinase C (PKC) and calcium-/calmodulin-dependent protein kinase II (CaMKII), requires local protein synthesis but not gene transcription (179). The following sustained LTP phase can last for hours and depends on both transcription and translation (167). Increased AMPA receptor density and dendritic spine remodeling (see below) represent widely accepted mechanisms for long-lasting LTP maintenance (154); yet, as recently pointed out (20), it has been difficult to reach consensus on how activation of NMDA receptors results in increased AMPA receptor density and dendritic spine remodeling. Evidence is emerging in relation to the signaling mechanisms that link synaptic activation, sustained LTP, and protein synthesis (36, 90, 114).

As detailed below (see Calcium-Dependent Gene Expression below), Ca\(^{2+}\) signals have a key role in connecting these processes. Future studies are bound to provide information on the complete array of proteins engaged in persistent changes in synaptic efficacy and on the cellular signaling pathways, including cross talk between Ca\(^{2+}\) and ROS signaling, underlying activity-mediated changes in protein synthesis.

**Structural Plasticity**

Dendritic spines are minute actin-rich dynamic protrusions present in most neurons that receive fast excitatory synaptic input in the brain. These remarkable structures, first described by Ramon y Cajal more than 100 years ago, have a variety of shapes and sizes (FIGURE 1B) and undergo significant remodeling during neuronal development and activity-dependent plasticity (214). Together with cytoskeleton remodeling and local protein synthesis, dendritic spine remodeling plays key roles in synaptic plasticity processes (42). As detailed in The Key Role of Ca\(^{2+}\) Signals in Synaptic Plasticity, activity-dependent Ca\(^{2+}\) signals play an essential role in hippocampal structural plasticity.

Spatial learning tasks that engage the hippocampus increase spine density in the CA1 region of trained rats (93, 132, 161, 162). Moreover, at hippocampal synapses, sustained LTP or LTD increase or decrease spine size, respectively (66, 89, 142, 154, 212, 218). The reported dendritic spine remodeling that goes along with LTP encompasses new spine growth, enlargement of preexisting spines and of their postsynaptic densities, and generation of two functional synapses via splitting of single postsynaptic densities and spines (1, 154, 214). Of note, whereas both spine shrinkage and LTD engage NMDA receptors and Ca\(^{2+}\)-mediated activation of calcineurin (also known as phosphatase 2B), spine shrinkage and LTD maintenance do not engage the same downstream pathways (218).

### The Key Role of Ca\(^{2+}\) Signals in Synaptic Plasticity

Soon after the discovery of LTP, several studies demonstrated the key role of postsynaptic Ca\(^{2+}\) signals in hippocampal synaptic plasticity (144, 148, 202, 209). Despite the fact that LTP strengthens while LTD weakens synaptic transmission, both responses require the generation of postsynaptic Ca\(^{2+}\) signals. The answer to the intriguing question of how intracellular [Ca\(^{2+}\)] increases generate these two opposing responses resides in the fact that Ca\(^{2+}\) signals that generate LTP or LTD exhibit different magnitudes and temporal courses. As illustrated in FIGURE 2, LTP-inducing tetanic stimulation generates, via strong depolarization, significant but short-lasting elevations of postsynaptic [Ca\(^{2+}\)] caused by robust activation of postsynaptic NMDA receptors (146, 150). In contrast, low-frequency stimulation produces a more moderate activation of NMDA receptors; the ensuing longer-lasting but modest postsynaptic [Ca\(^{2+}\)] increases are optimal for LTD induction (50, 142). Whereas the high-amplitude Ca\(^{2+}\) signals underlying hippocampal LTP at the CA1 region require Ca\(^{2+}\) influx through postsynaptic NMDA receptors (150), additional sources, including L-type voltage-gated Ca\(^{2+}\) channels and extrasynaptic NMDA receptors, contribute to the low but more sustained Ca\(^{2+}\) increase leading to LTD (153).

### Calcium-Dependent Signaling Pathways

The brief Ca\(^{2+}\) influx generated by activation of postsynaptic NMDA receptors produces local
Ca\(^{2+}\) signals, which, depending on their extent and magnitude, engage a variety of intracellular pathways. Among other effects, postsynaptic intracellular Ca\(^{2+}\) signals promote AMPA receptor accumulation by decreasing their diffusion and thus increasing their dwell time at individual synapses (32, 74, 195). Likewise, inhibitory scaffolds and the GABAA receptor display calcium-dependent changes in synaptic trafficking, both of which are synaptic activity-modulated (17). Calcium influx via NMDA receptors also promotes the generation of small-messenger molecules such as arachidonic acid, cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate, and nitric oxide (156, 194), all of which engage specific neuronal signaling cascades.

Current evidence indicates that Ca\(^{2+}\)-dependent activation of serine/threonine protein kinases and phosphatases has a central role in the LTP/LTD responses. As stated above, postsynaptic Ca\(^{2+}\) influx via NMDA receptors is critical for LTP induction (179). The Ca\(^{2+}\) binding protein calmodulin (CaM) senses postsynaptic Ca\(^{2+}\) signals generated via activation of NMDA receptors or L-type voltage-dependent Ca\(^{2+}\) channels. The resultant Ca\(^{2+}\)/CaM complex initiates a chain of signaling events, including activation of Ca\(^{2+}\)/CaM-dependent kinase II (CaMKII), which facilitates LTP. In contrast, calcium-dependent activation of phosphatases such as calcineurin (175) facilitates LTD. Biophysical models indicate that the higher frequencies (shorter durations, higher amplitudes) used to induce LTP (FIGURE 2) are more efficient to activate CaMKII, whereas the lower frequencies (lower amplitude, longer durations) that promote LTD facilitate phosphatase activation (133).

Both CaMKII and the extracellular signal-regulated kinases ERK1/2, which belong to the mitogen-activated kinase (MAPK) family, have critical roles in the signaling cascades initiated by Ca\(^{2+}\) influx mediated by activated NMDA receptors. All CaMKII isoforms undergo activation by the Ca\(^{2+}\)/CaM complex; the resulting activated CaMKII enzyme can sustain its own activity through autophosphorylation (158). This property led John Lisman and collaborators to propose that CaMKII has the potential to function as a local, self-perpetuating memory molecule (137, 138). In effect, mice carrying a CaMKII mutation that prevents its autophosphorylation fail to exhibit LTP induction in the hippocampal CA1 region and exhibit significant deficits in hippocampus-dependent learning and memory tasks (69). Calcium also plays a central role in NMDA receptor-dependent activation of the ERK1/2 cascade (84, 102, 122), whereas ERK1/2 inhibition prevents LTP maintenance and long-term memory formation (9, 29, 35). Presently, ERK1/2 phosphorylation is considered critical for sustained LTP maintenance and memory consolidation and storage (114, 176, 199). The calcium-mediated LTD response engages protein dephosphorylation by two calcium-responsive

![FIGURE 2. Frequency-dependent Ca\(^{2+}\) signals, synaptic strength, and post-burst action potentials in young and aged neurons](http://physiologyonline.physiology.org/)

A: the amplitude and the time course of activity-generated Ca\(^{2+}\) signals vary according to stimulation frequency. B: frequency-dependent changes in synaptic strength displayed by the CA1 region of hippocampal slices from young animals. C: frequency-dependent changes in synaptic strength displayed by the hippocampal CA1 region of slices from aged animals. D: post-burst action potential recorded in CA1 neurons from young rodents (blue trace). Treatment with xanthine/xanthine oxidase (X/XO) increases the slow AHP phase (red trace). E: post-burst action potential recorded in CA1 neurons from aged rodents (red trace), showing a long-lasting slow AHP phase. The antioxidant DTT or RyR inhibition with ryanodine drastically decreases this prolonged phase (blue trace).
phosphatases, calcineurin and protein phosphatase 1 (PP1) (136). As described above, LTD induction requires a modest [Ca\textsuperscript{2+}] increase that is likely to activate calcineurin, which has significantly higher affinity for Ca\textsuperscript{2+} than CaMKII. Albeit current evidence supports the role of calcineurin and PP1 in LTD, this response is likely to involve other cellular signaling pathways, including site-specific phosphorylation of AMPA receptors (45), and possibly modulation of calcineurin activity by changes in cellular redox state, as detailed below. Furthermore, recent findings indicate that LTP and LTD both require CaMKII activation (47), a further indication of the complexity of the Ca\textsuperscript{2+} signaling pathways underlying LTP and LTD.

**Calcium Release Channels**

Whereas the contribution of activity-dependent Ca\textsuperscript{2+} influx mediated by NMDA receptors to neuronal plasticity processes is widely acknowledged, the involvement of Ca\textsuperscript{2+} signals generated by Ca\textsuperscript{2+} release from the endoplasmic reticulum (ER) remains less recognized. Early studies showed that inhibition with thapsigargin of the sarco-/endoplasmic reticulum Ca\textsuperscript{2+} (SERCA) pump, which leads to irreversible Ca\textsuperscript{2+} depletion from the ER, blocks LTP induction in rat hippocampal slices but does not affect NMDA receptor-dependent currents (85) and also prevents LTD induction (182). Likewise, pre-incubation with dantrolene, an inhibitor of Ca\textsuperscript{2+} release from the ER mediated by ryanodine receptor (RyR) channels, significantly reduces NMDA receptor initiated Ca\textsuperscript{2+} signals (191), indicating that at least part of the postsynaptic Ca\textsuperscript{2+} rise originates from intracellular release of Ca\textsuperscript{2+}. Dantrolene also prevents LTP induction by one train of HFS (170), whereas inhibition of RyR-mediated Ca\textsuperscript{2+} release with inhibitory concentrations of ryanodine prevents LTD induction by the TBS protocol (180, 181). In contrast, LTD induction by four trains of HFS does not require RyR-mediated Ca\textsuperscript{2+} release (141). These combined results suggest that RyR-mediated Ca\textsuperscript{2+} release contributes to LTD induction only when moderate tetanic stimulation protocols are used. Presumably, the more robust protocols represented by multiple trains of HFS generate postsynaptic Ca\textsuperscript{2+} signals of sufficient magnitude to stimulate the Ca\textsuperscript{2+}-dependent signaling cascades responsible for LTP induction. Sustained LTD induction by four trains of HFS, however, does require RyR-mediated Ca\textsuperscript{2+} release, whereas RyR activation converts early LTP into late LTD (141). These findings strongly suggest that RyR-mediated Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release (CICR) is essential for triggering the transcription and translation processes underlying the sustained LTD phase (167). In contrast, several reports indicate that type-1 inositol 1,4,5-trisphosphate (IP\textsubscript{3}) receptors negatively regulate the induction of LTP (reviewed in Ref. 174).

Other studies have implicated Ca\textsuperscript{2+} release from separate presynaptic and postsynaptic intracellular stores in the induction of hippocampal LTD in the CA3-CA1 synapse (182). In particular, Ca\textsuperscript{2+} release from internal stores has a role in the control of LTD in the CA3-CA1 synapse (168). Of note, more recent studies showed that low concentrations of the RyR agonists caffeine and ryanodine (which at low concentrations acts as a RyR agonist) do not modify the paired-pulse response in CA3-CA1 hippocampal synapse but enhance LTD induction (73), suggesting that RyR-mediated Ca\textsuperscript{2+} release contributes to postsynaptic rather than presynaptic responses. In addition, caffeine-induced activation of RyR-mediated Ca\textsuperscript{2+} release from the ER stimulates dendritic spine remodeling (120). The spine remodeling induced by brain-derived neurotrophic factor (BDNF) also requires RyR-mediated Ca\textsuperscript{2+} release (2). Two recent reviews (16, 174) present a more detailed description of the role of Ca\textsuperscript{2+} release from the ER in synaptic plasticity processes.

**Calcium-Dependent Gene Expression**

Calcium-dependent changes in gene expression induced by neuronal activity elicit a wide range of processes and behaviors, including persistent synaptic plasticity, dendritic structural changes, and memory consolidation (76, 206). Activity-generated neuronal Ca\textsuperscript{2+} signals promote gene expression by stimulating diverse signaling cascades, including induction of de novo DNA methylation that, via global chromatin remodeling, modifies gene transcription (76). In particular, through dephosphorylation by calcium-activated calcineurin, cytoplasmic Ca\textsuperscript{2+} signals stimulate translocation to the nucleus of calcineurin and nuclear factor of activated T-cells (NFAT), where it promotes transcription (71, 91).

**Nuclear Ca\textsuperscript{2+} Signals**

Robust stimulation of hippocampal neurons generates postsynaptic Ca\textsuperscript{2+} signals that propagate to the cell nucleus, where they have key roles in the regulation of gene expression and perform a particularly important role in activity-dependent transcription (10, 76, 81, 83, 217). Nuclear Ca\textsuperscript{2+}/CaM signaling has a crucial role in neuronal activity-induced gene transcription and transcription-dependent LTP and long-term memory (135). In particular, nuclear Ca\textsuperscript{2+} signals activate protein kinases, leading to phosphorylation/activation and/or changes in the localization of a variety of transcriptional regulators (10). Calcium-activated
nuclear kinases target diverse transcription factors, including cyclic AMP response element binding protein (CREB), ternary complex factor (TCF)/serum response factor (SRF) transcription factor complex, nuclear factor-κB (NF-κB), and myocyte enhancer factor 2 (MEF2), among others (12, 48, 72, 211). In addition, nuclear Ca^{2+} signals directly activate the transcription factor downstream regulatory element antagonistic modulator (DREAM) (40, 128), which exerts a negative control on CREB-dependent gene transcription, LTP, and transcription-dependent long-term memory (4, 61, 134). Nuclear Ca^{2+} signals stimulate CREB binding protein (CBP) via phosphorylation by CaMKIV (51, 82, 97), a protein kinase with constitutive nuclear localization (25, 101, 166). Activation of CaMKIV requires, in turn, binding of nuclear Ca^{2+}/CaM (7, 80), plus phosphorylation by Ca^{2+}/CaM-dependent protein kinase kinase (CaMKK) (6, 7, 54, 155, 189). Activated CBP, which is a histone acetyltransferase, promotes transcription via physical interaction with numerous transcription factors, including CREB, and influences gene transcription by catalyzing histone acetylation and subsequent chromatin decondensation (3, 22, 51, 121). Nuclear Ca^{2+} signals also promote the subcellular redistribution of class II histone deacetylases (HDACs), which, via chromatin compression, limit the accessibility of transcription factors to their target binding sequences (51, 200, 201). In addition to Ca^{2+} signals generated via NMDA receptor activation, voltage-dependent Ca^{2+} channels contribute to neuronal activity-dependent regulation of gene expression (72, 103, 177, 180, 181). The transcription factor CREB modulates many functions of the central nervous system, including synaptic plasticity, memory formation, and neurogenesis (34, 35, 52, 100, 175). CREB-mediated transcription requires persistent CREB phosphorylation, mediated by the Ras/ERK1/2 signaling pathway and CBP activation by nuclear Ca^{2+} signals (11). Synaptic NMDA receptor activation induces CREB phosphorylation via the joint action of nuclear CaMKIV, which mediates rapid CREB phosphorylation, and the Ras/ERK1/2 pathway that promotes slower but more long-lasting CREB phosphorylation (81, 210). Hippocampal LTP induction promotes calcium-dependent CREB phosphorylation (55, 99), a requisite step for gene expression dependent on the cAMP-response element (CRE) that underlies sustained LTP (98, 140, 205). Calcium-dependent activation of the nuclear CREB/CBP complex enhances transcription of BDNF, a protein with a central role in long-term synaptic/structural plasticity and hippocampal-dependent memory processes (37). Activity-induced BDNF expression in the hippocampus induces sustained synaptic and structural plasticity (174), which most likely underlie some forms of long-term memory.

Induction of calcium-dependent gene transcription in the nucleus following synaptic activation requires that local Ca^{2+} signals reach the nucleus to modify gene expression. The cellular mechanisms underlying propagation of activity-dependent Ca^{2+} signals to the nucleus from their sites of generation at postsynaptic spines, dendrites, or the soma represent an unresolved problem of present-day neuroscience. The diffusion of Ca^{2+} ions in the cytoplasm is highly restricted by the presence of abundant cytoplasmic Ca^{2+} buffers (5). Hence, neurons must use other mechanisms to propagate activity-dependent Ca^{2+} signals to the nucleus (76). Diffusion and/or active transport and subsequent nuclear translocation of Ca^{2+}-regulated proteins from their synaptic sites of activation into the nucleus may give rise to nuclear Ca^{2+} signals (56, 71, 104, 126, 157, 173, 208, 216). Alternatively, propagation of internal Ca^{2+} release waves mediated by IP_{3} receptors to the somato-nuclear compartment represents a second possible mechanism (77, 80, 178, 204). A third hypothetical mechanism of Ca^{2+} signal propagation to the nucleus (23) considers the generation of propagated Ca^{2+} waves via RyR-mediated CICR. In fact, activity-induced Ca^{2+} influx via the NMDA receptor in dendritic spines undergoes significant amplification by RyR-dependent CICR (58, 181). Despite reports showing that synaptic NMDA receptor-dependent Ca^{2+} signals remain confined to the spine heads (165, 169, 188, 215), a study showing that stimulation of synaptic NMDA receptors requires Ca^{2+} release from stores to generate the nuclear Ca^{2+} signals underlying CREB/CBP-mediated transcription supports this third hypothesis (80).

**ROS in Synaptic Plasticity**

Current evidence indicates that at physiological levels ROS act as cellular second messengers by promoting reversible redox modifications of different cellular components, including oxidative modifications of protein cysteine residues with low pKa (88, 171). The brain has a very active oxidative metabolism compared with other organs (79). Neuronal activity generates considerable ROS levels, and it is becoming increasingly apparent that this ROS increase plays key roles in the functional and structural changes that mediate hippocampal synaptic plasticity and hippocampus-dependent memory formation (152). In particular, several protein molecules relevant for LTP and LTD undergo reversible redox modifications (FIGURE 3), such as NMDA receptors, Ca^{2+}-activated K^{+} channels, IP_{3} receptors, RyR channels, the protein phosphatase...
calcineurin (88), and the protein kinase CaMKII (30), which impinge on their physiological function. Accordingly, preserving cellular redox balance is essential for safeguarding synaptic plasticity, among other neuronal functions. To avoid oxidative stress and the ensuing damage to cellular components due to activity-generated ROS production, neurons maintain their redox balance by means of antioxidant enzymes that include superoxide dismutase (SOD), catalase, and glutathione (GSH) peroxidases (171).

Activation of NMDA receptors in rat hippocampal pyramidal neurons in culture and in brain slices stimulates superoxide anion production (24), which in turn promotes ERK1/2 phosphorylation (111). In mixed hippocampal cultures, Ca^{2+}-influx induced by glutamate enhances ROS generation in neurons but not in astrocytes (106). Several sources, including mitochondria, are responsible for cellular ROS generation. Among them, the highly regulated NADPH oxidases (38) are important generators of the ROS species involved in signaling cascades because these enzymes exhibit rapid activation and inactivation kinetics, allowing transient increases in cellular ROS levels (190). In particular, enzymatic ROS generation via the NOX2 isoform (a member of the NADPH oxidase family) causes the activation of ERK1/2 in hippocampal area CA1 (117). In hippocampal neurons, NOX2 is present at synaptic locations (196), where it undergoes activation via stimulation of postsynaptic neuronal nitric oxide synthase by NMDA receptor-mediated Ca^{2+} influx (70).

Superoxide anion is a free radical that undergoes rapid spontaneous or SOD-mediated enzymatic dismutation to H_{2}O_{2}, a cellular ROS directly responsible for NMDA receptor-dependent activation of ERK1/2 in hippocampal area CA1 (111). In hippocampal neurons, NOX2 is present at synaptic locations (196), where it undergoes activation via stimulation of postsynaptic neuronal nitric oxide synthase by NMDA receptor-mediated Ca^{2+} influx (70).

ROS and LTP

Several reports indicate that ROS play key roles in hippocampal LTP formation and maintenance (116, 152). Scavenging superoxide with manganese porphyrin compounds that mimic SOD function prevents HFS-induced LTP in hippocampal slices (118), whereas overexpressing in mice the three different mammalian SOD isoforms affect LTP in different ways (152). Low hippocampal superoxide
levels in mice overexpressing extracellular SOD (EC-SOD) cause impaired NMDA receptor-dependent LTP in the CA1 region (198); increased H₂O₂ generation produced by superoxide dismutation in mice overexpressing the cytoplasmic SOD-1 isoform has the same effect (109). In contrast, transgenic mice that overexpress the mitochondrial SOD isoform (SOD-2) exhibit normal LTP (94), strongly suggesting that mitochondria-generated superoxide anion does not contribute to LTP induction and maintenance. Generation of superoxide in vitro via xanthine/xanthine oxidase induces LTP in hippocampal area CA1 by acting downstream of NMDA receptors; superoxide-induced LTD occludes HFS-induced LTP, suggesting recruitment of the same signaling pathways (119). LTP induction fails in mutant mice lacking NOX2 subunits, which also display mild deficits in hippocampus-dependent memory tasks (116). Since ERK activation is required for NMDA receptor-dependent LTP (59), these combined results suggest that NOX2-generated ROS lead to Ca²⁺-dependent ERK1/2 activation during hippocampal LTP.

The second messenger role of hydrogen peroxide is emerging (183). Dismutation of superoxide radicals generates hydrogen peroxide in hippocampal neurons, which affects LTP in complex ways. In rat hippocampal slices, H₂O₂ causes initial augmentation but subsequent long-lasting depression of field excitatory postsynaptic potentials (fEPSP); the initial fEPSP increase is not affected by NMDA receptor inhibition or by iron chelation, indicating that NMDA receptor-dependent Ca²⁺ influx or hydroxyl radical generation does not mediate the stimulatory effects of H₂O₂ (113). A subsequent study showed that low H₂O₂ concentrations potentiate whereas higher concentrations depress HFS-induced LTP (109). Transgenic mice overexpressing SOD-1, which maintain high endogenous H₂O₂ levels and display impaired hippocampal LTP, exhibit enhanced TBS-induced LTP at H₂O₂ concentrations that block LTP in wild-type mice (108). The authors concluded that these seemingly paradoxical effects of H₂O₂ were specific to H₂O₂ because in vitro superoxide generation by X/XO did not mimic these effects; moreover, they proposed that SOD-1 transgenic mice are less sensitive to activity-induced ROS generation and require higher H₂O₂ levels (detrimental to wild-type mice) for LTP induction by TBS (109). This suggestion predicts that neuronal H₂O₂ levels increase following TBS and that the ambient H₂O₂ levels determine the magnitude of this increase by conditioning the activity of cellular antioxidant systems. In addition to H₂O₂, iron-generated ROS also stimulate LTP. Iron addition to primary hippocampal neurons rapidly increases the intracellular labile iron pool and stimulates ROS production; moreover, iron addition to rodent hippocampal slices facilitates sustained LTP induction (CA3 to CA1) after suboptimal tetanic stimulation, whereas iron chelation decreases basal synaptic transmission and prevents sustained TBS-induced LTP (163).

**ROS and LTD**

To our knowledge, there is no information regarding redox modulation of hippocampal LTD. Since superoxide anion inhibits calcineurin activity (187), which as stated above is a protein phosphatase essential for LTD, activity-generated ROS may have a negative effect on LTD induction. This prediction needs experimental testing, however, because Ca²⁺ and ROS exert opposite effects on calcineurin activity. Accordingly, LTD induction would decrease, provided inhibition of calcineurin activity by ROS prevailed over Ca²⁺-induced stimulation.

**Cross Talk Between Calcium and ROS: Implications for Aged-Related Defects in Synaptic Plasticity**

Growing evidence indicates that there is significant cross talk between Ca²⁺ and ROS signaling in numerous cellular processes (88). In cultured hippocampal neurons, H₂O₂ prompts RyR-mediated Ca²⁺ release that stimulates ERK/CREB phosphorylation (115) and transcription of early genes (87). In agreement, a later study also reported that H₂O₂ releases Ca²⁺ from the ER in primary hippocampal cultures (67). High-frequency field stimulation (HFFS) of primary hippocampal neurons generates hydrogen peroxide by stimulating NOX2 via NMDA receptor-mediated Ca²⁺ entry and RyR-mediated CICR, and increases type 2 RyR (RyR2) protein levels, whereas the antioxidant agent N-acetylcysteine (NAC), a precursor of cellular glutathione generation, prevents these responses (184). These results suggest that the joint increase in Ca²⁺ and ROS produced by HFFS promotes RyR2 expression. Furthermore, superoxide anion, in addition to promoting ERK phosphorylation and potentiation of synaptic transmission in area CA1, increases [³H]-ryanodine binding in hippocampal cell extracts, indicating enhanced RyR activity (96). Superoxide-induced potentiation requires functional L-type Ca²⁺ channels and does not occur in knockout mice for type 3 RyR (RyR3) channels, suggesting that this particular form of ROS-induced potentiation requires RyR3-mediated Ca²⁺ release (96).

As mentioned in previous sections, the increases in both cytoplasmic Ca²⁺ and ROS levels produced by neuronal activity play essential roles as signaling elements in hippocampal synaptic plasticity.
Cross talk between Ca$^{2+}$ and ROS is likely to occur during synaptic plasticity induction and maintenance, but excessive ROS production may produce negative effects. In fact, high cellular ROS levels caused by excessive ROS production, by less-effective cellular antioxidant systems or by a combination of both factors, promote oxidative stress, a key feature of aged-related impairments in synaptic plasticity and cognitive functions. Antioxidant agents may help to restore physiological ROS levels, albeit animal studies using antioxidant agents have yielded inconclusive results, presumably caused by their inactivation during intestinal absorption (78).

Alterations in Ca$^{2+}$ and ROS cross talk most probably occur during aging, which entails neuronal oxidative stress (174) and dysfunctional neuronal Ca$^{2+}$ signaling (172). During aging, enhanced ROS generation may contribute to reduce the function of NMDA receptors and stimulate Ca$^{2+}$ signals.

**FIGURE 4.** LTP responses in young and old rodents

A: LTP responses recorded in acute hippocampal slices from young (blue traces) and aged animals (red traces), before (control) or after treatment with NAC. B: LTP responses recorded in acute hippocampal slices from young (blue traces) or aged animals (red traces) after chronic treatment with NAC. Figure is modified from Ref. 86 with permission from John Wiley & Sons, Ltd.
generated by CICR mediated by redox-modified RyR channels, which occurs in response to Ca\(^{2+}\) entry via L-type voltage-gated Ca\(^{2+}\) channels (62, 174). This ROS-enhanced CICR may produce abnormal increments of intracellular Ca\(^{2+}\) levels that, if uncontrolled, are bound to promote neuronal death (53). Treatment with dithiothreitol (DTT) of hippocampal slices from aged rats enhances NMDA receptor responses by enhancing CaMKII activity; however, DTT either had no effect or decreased CaMKII activity in CA1 from young animals (30). Therefore, a shift in intracellular redox state to more oxidative conditions in aged rodents may contribute to the decline in NMDA receptor responses through CaMKII-mediated signaling.

Aged animals display hippocampal LTP impairments (19, 123). As illustrated in FIGURE 2C, aged rodents display decreased LTP but enhanced LTD compared with young animals. Moreover, compared with the post-burst action potential response of young rodents (FIGURE 2D), aged rodents present an abnormally long-lasting slow after hyperpolarization (AHP) phase (FIGURE 2E) that results in decreased excitability of CA1 pyramidal neurons (124). This increase in the slow AHP phase is due to activation of Ca\(^{2+}\)-activated K\(^+\) channels by stimulation of RyR-mediated Ca\(^{2+}\) release (124), since inhibitory ryanodine suppresses the prolonged slow AHP phase (FIGURE 2E). In the context of Ca\(^{2+}\)-ROS signaling cross talk, it is worth highlighting that the reducing agent DTT significantly decreases the slow AHP component in CA1 pyramidal neurons of aged rats (FIGURE 2E) but does not affect the action potential response of young rats (31). Altogether, these results indicate that the increased oxidative tone exhibited by neurons during aging promotes RyR-mediated CICR release in response to post-burst stimulation. The enhancement of the slow AHP phase produced by treatment of CA1 pyramidal neurons with the superoxide generating X/O system illustrated in FIGURE 2D (31) supports this proposal. In addition, RyR-mediated Ca\(^{2+}\) release from the ER differentially modifies LTP in young and old animals. Thus RyR inhibition with ryanodine prevents LTP induction in hippocampal slices from young rats (180) but facilitates LTP induction in hippocampal slices from old rats (124).

Loss of the inhibitory effects of FKBP12.6/1b, a protein that binds and stabilizes the cardiac RyR2 channels in the closed state, results in Ca\(^{2+}\) signaling dysregulation in the hippocampus (64). This finding is relevant for age-related neuronal dysfunction, since leaky neuronal RyR2 channels seemingly underlie stress-induced cognitive dysfunction (139). Moreover, delivery of a transgene encoding the FKBP12.6/1b protein to the hippocampus decreases the slow AHP phase displayed by neurons of aged hippocampus (63). Albeit there is no information regarding the effects of RyR2
oxidation on its interaction with the FKBP12.6/1b protein, oxidation of type 1 RyR channels decreases the affinity of FKBP12 binding to these channels (8).

The aged brain displays an increase in oxidative damage and a decrease in GSH levels and redox buffering capacity (174). The oxidative stress conditions associated to the aging process may contribute to impair LTP; in fact, overexpression of EC-SOD or SOD-1 throughout the lifetime of mice protects against age-related LTP deficits (95). Aged hippocampal neurons display higher ROS levels, which reduce NMDA receptor function (197, 203); these combined factors produce a shift from NMDA receptor-dependent LTP to LTP that depends on Ca2+ entry via L-type Ca2+ channels (33, 185). Furthermore, RyR channels also contribute to enhance LTD during aging, since inhibition of RyR-mediated Ca2+ release from the ER prevents LTD induction in aged rats (125).

Of note, as illustrated in (FIGURE 4A), acute slices from young mice incubated with NAC display a reduction of sustained LTP induction in the CA region following stimulation of the Schaffer collateral pathway, whereas acute slices from aged mice display reduced LTP induction and maintenance but increased LTP after NAC incubation (86). In addition, chronic NAC feeding prevents oxidative damage in the hippocampus of aged rats; it also produces a significant stimulation of both LTP induction and maintenance in aged mice (FIGURE 4B) and reverses the L-type Ca2+ channel-dependent LTP seen in aged animals to NMDA receptor-dependent LTP (86). Interestingly, H2O2 reverses the LTP impairments displayed by acute slices from aged mice but reduces LTP in slices from aged SOD-1 transgenic mice, which in the absence of H2O2 exhibit enhanced LTP responses compared with aged wild-type mice (109). These results indicate a dual role for H2O2 in the regulation of LTP in aged mice, presumably mediated by differential modulation of the protein phosphatase calcineurin by the different basal ROS levels of wild-type and SOD-1 transgenic mice (109).

To sum up the above results, we propose the model illustrated in FIGURE 5. The increased post-synaptic ROS levels in CA1 neurons from aged compared with young rodents, represented by the deeper orange color of the cytoplasm, induce RyR oxidative modifications that stimulate RyR-mediated Ca2+ release in response to activity-induced Ca2+ entry. Moreover, CA1 neurons from aged rodents express more L-type Ca2+ channels (197, 203) and more type 2 RyR channels (Arias-Cavieres A, Muñoz P, Hidalgo C, unpublished observations) than neurons from young animals. These combined features produce increased Ca2+ signals in aged compared with young neurons, leading to the enhanced slow AHP produced by activation of Ca2+-activated K+ channels, which results in decreased neuronal excitability and frequency-decoding capacity.

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