Themed Section: Pharmacology of the Gasotransmitters

REVIEW

S-nitrosation and neuronal plasticity

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Nitric oxide (NO) has long been recognized as a multifaceted participant in brain physiology. Despite the knowledge that was gathered over many years regarding the contribution of NO to neuronal plasticity, for example the ability of the brain to change in response to new stimuli, only in recent years have we begun to understand how NO acts on the molecular and cellular level to orchestrate such important phenomena as synaptic plasticity (modification of the strength of existing synapses) or the formation of new synapses (synaptogenesis) and new neurons (neurogenesis). Post-translational modification of proteins by NO derivatives or reactive nitrogen species is a non-classical mechanism for signalling by NO. S-nitrosation is a reversible post-translational modification of thiol groups (mainly on cysteines) that may result in a change of function of the modified protein. S-nitrosation of key target proteins has emerged as a main regulatory mechanism by which NO can influence several levels of brain plasticity, which are reviewed in this work. Understanding how S-nitrosation contributes to neural plasticity can help us to better understand the physiology of these processes, and to better address pathological changes in plasticity that are involved in the pathophysiology of several neurological diseases.

LINKED ARTICLES

This article is part of a themed section on Pharmacology of the Gasotransmitters. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2015.172.issue-6

Abbreviations

BDNF, brain-derived neurotrophic factor; CaMKII, calcium/calmodulin-dependent protein kinase II; Cdk5, cyclin-dependent kinase 5; cGMP, cyclic GMP; CREB, cAMP response element-binding protein; DRG, dorsal root ganglion; Drp1, dynamin-related protein 1; eNOS, endothelial NOS; EPSP, excitatory postsynaptic potential; HDAC2, histone deacetylase 2; iNOS, inducible NOS; LTD, long-term depression; LTP, long-term potentiation; nNOS, neuronal NOS; NSC, neural stem cells; NSF, N-ethyl-maleimide sensitive factor; PSD-95, postsynaptic density protein 95; PTM, post-translational modifications; RNS, reactive nitrogen species; RyR, ryanodine receptor; sGC, soluble guanylate cyclase

Table of Links

TARGETS			LIGANDS
AMPA receptors	GluA1	HDAC2, histone deacetylase 2	BDNF
CaMKII, calmodulin-dependent protein kinase II	GluA2	NMDA receptors	cGMP
Cdk5, cyclin-dependent kinase 5	GluA3	NOS, NO synthase	GSH
CREB	GluN1	PKG, protein kinase G	NO
EGF receptor	GluN2A	RyR1, ryanodine receptor 1	
ERK	sGC, soluble guanylyl cyclase		

This Table lists key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 (Alexander *et al.*, 2013a, b, c d).

Introduction

Brain plasticity is regarded as the ability of the brain to change its structure and function as a result of experience and changes in environment. Plasticity occurs mostly at three different levels: modification of the strength of existing synapses (synaptic plasticity), formation of new synapses (synaptogenesis) and formation of new neurons (neurogenesis). Many factors, including nitric oxide (NO), regulate and fine-tune these mechanisms.

Synaptic plasticity is described as the change in strength of a synapse in response to changes in synaptic activity. Short-term plasticity occurs on a scale from milliseconds to minutes, and is often associated with changes in the presynaptic release of neurotransmitters. Longer forms of plasticity occur in the postsynaptic terminal, such as long-term potentiation (LTP) or depression (LTD); initial changes (in the first 1 or 2 h) in LTP or LTD are associated with alterations in the trafficking of glutamate receptors, while maintenance of LTP and LTD for longer periods requires changes in gene expression. The increase in postsynaptic strength is triggered by activation of NMDA receptors, which causes calcium influx into the postsynaptic cell and activates signal transduction pathways that result in insertion in the membrane of a different type of glutamate receptors, AMPA receptors. The regulation of membrane insertion of AMPA receptors is key for efficient synaptic plasticity, and a large body of evidence can be found regarding the control of AMPA receptor trafficking (see Ho *et al.*, 2011b), including by NO.

Synaptogenesis refers not only to formation of new synapses, but also to the remodelling of existing ones. Synapse formation occurs during development and in the adult brain, and may be changed by experience or disease (see Pérez-Domper *et al.*, 2013; Robichaux and Cowan, 2014). NO has been consistently involved in such a rearrangement of existing synapses and formation of new ones (García-López *et al.*, 2010; Shelly *et al.*, 2010; Sunico *et al.*, 2010).

Neurogenesis is not restricted to embryonic development, but occurs throughout adult life. New neurons and glial cells are continuously formed in the brain, from neural stem cells present in niches such as the subventricular zone and the dentate gyrus in the hippocampus (see Gage and Temple, 2013). Neurogenesis is a tightly regulated process, and NO is one of the many factors that influence the formation of new brain cells in physiological conditions and in disease states (see Carreira *et al.*, 2012; Estrada and Murillo-Carretero, 2005).

In the brain, the free radical gaseous messenger NO is associated with the regulation of neuronal plasticity and cognitive functions, and also regulates biological functions such as the sleep-wake cycle, body temperature, appetite and hormone release, and modulates neuronal development, nociception and apoptosis (see Calabrese *et al.*, 2007; Prast and Philippu, 2001). NO is mainly produced by nitric oxide synthases (NOS), which can be found in the CNS in three different isoforms: (i) neuronal NOS (nNOS or NOS1), which is constitutively expressed in neurons; (ii) endothelial NOS (eNOS or NOS3), constitutively expressed in endothelial cells; and (iii) inducible NOS (iNOS or NOS2), which is mainly expressed by activated microglial cells. While nNOS and eNOS are constitutively expressed and are transiently activated by calcium/calmodulin and other regulators, iNOS is transcriptionally regulated by inflammation or toxic stimuli and its activity is independent of calcium regulation (Bredt, 1999; Guix *et al.*, 2005).

The potential of NO as a modulator of synaptic transmission was first proposed in 1988 (Garthwaite *et al.*, 1988), and its presence was documented in several brain regions such as the hippocampus, striatum, hypothalamus and locus coeruleus (Prast and Philippu, 2001). The small size of NO, its highly diffusible nature and its link to glutamate receptors make NO a special neurotransmitter. NO may be considered an atypical neurotransmitter, as it does not require vesicle storage or exocytotic release, being produced generally after receptor activation and readily diffused throughout cells and tissues. NO also modulates the release of several neurotransmitters such as acetylcholine, catecholamines, glutamate and GABA (see Prast and Philippu, 2001). NO is able to mediate both postsynaptic intracellular signalling and retrograde intercellular communication between different types of cells and tissues. However, the short half-life of NO and its diffusion coefficient (2.2×10^{-5} cm^2 s^{-1} obtained *in vivo*) limit the range of action of NO in brain tissue, thus providing short-range responses in individual synapses (Santos *et al.*, 2011; Martínez-Ruiz *et al.*, 2013).

Classical NO signalling proceeds through its potent binding to soluble guanylate cyclase (sGC), which activates this enzyme that produces cyclic GMP (cGMP). cGMP in turn reacts with a family of cGMP-dependent protein kinases (also called PKG), triggering several signalling pathways (see Hofmann, 2005; Francis *et al.*, 2010). A less classical mode of action of NO is through interaction with mitochondrial proteins, especially inhibition of cytochrome *c* oxidase. Nonclassical NO signalling includes both the production of NO from alternative sources (mainly nitrite) and the formation of covalent post-translational modifications (PTM) in proteins (see Martínez-Ruiz *et al.*, 2011). These PTM are not formed by NO itself, but by the production of the so-called reactive nitrogen species (RNS), small molecules produced from NO reactions. Although there are several RNS-induced PTM, three of them have been more intensively studied. Tyrosine nitration, the incorporation of a nitro group ($-NO_2$) to a tyrosine residue, is considered an almost irreversible modification, so it has been related to protein damage, although it is also implied in signalling pathways (Souza *et al.*, 2008; Ischiropoulos, 2009). Cysteine S-glutathionylation, the formation of a mixed disulfide between a protein cysteine thiol and glutathione, is a reversible modification whose formation and removal may be mediated by enzymes such as glutaredoxins. It has been involved in cell signalling and in oxidative stress, it can be formed from either reactive oxygen species or RNS, and it is related to S-nitrosation in several ways, including the fact that S-nitrosation of a cysteine residue or of glutathione can lead to S-glutathionylation (see Martínez-Ruiz and Lamas, 2007; Mieyal *et al.*, 2008; Sabens Liedhegner *et al.*, 2012).

Protein S-nitrosation is the formation of a nitrosothiol (R-S-NO) in cysteine residues (denoted PSNO). It is also called S-nitrosylation (the terminology has been discussed in Martínez-Ruiz and Lamas, 2004; Heinrich *et al.*, 2013), and both names could be used for the overall description of the PTM, as their difference relies mainly in the chemical mechanisms for their formation, which is not always known or considered (Heinrich *et al.*, 2013). We will follow the recent recommendation in this Journal to use the term S-nitrosation as it includes the most part of the cases and can induce less confusion (Heinrich *et al.*, 2013). Nitrosothiols are not formed from the direct reaction of NO with the cysteine residue, unless it is in the thiyl radical form (P-S•).

$$P\text{-}S\bullet + \bullet NO \rightarrow P\text{-}S\text{-}NO$$
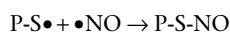
There are several chemical mechanisms that can lead to protein S-nitrosation, including formation of N_2O_3,

$$2\,NO + O_2 \rightarrow 2\,NO_2$$

$$NO_2 + NO \rightarrow N_2O_3$$
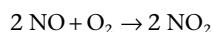
a species that is a very effective nitrosating agent.

$$N_2O_3 + PSH \rightarrow PSNO + H^+ + NO_2^-$$

Nitrosothiols are not very stable in the cells compared with other PTM because of the lability of the bond, the presence of denitrosylases and other reductants (e.g. ascorbate), and the easy reaction with other thiols to transfer the nitroso group to either low molecular mass thiols or proteins (transnitrosation)

$$PSNO + RSH \rightarrow PSH + RSNO$$

$$PSNO + P'SH \rightarrow PSH + P'SNO$$
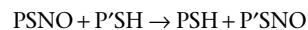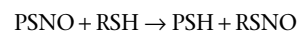
or to produce a mixed disulfide, leading to S-thiolation or S-glutathionylation.

$$PSNO + RSH \rightarrow PSSR + HNO \text{ (S-thiolation: RSH is a low molecular mass thiol)}$$

$$PSNO + GSH \rightarrow PSSG + HNO \text{ (S-glutathionylation: GSH is glutathione).}$$
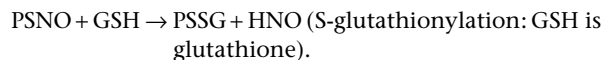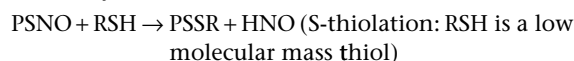
Enzymic catalysis is not strictly necessary for its formation and degradation in cell environment, so its specificity and involvement in cell signalling bears some properties that make it different from other signalling PTM such as phosphorylation (see Martínez-Ruiz and Lamas, 2004; Martínez-Ruiz *et al.*, 2011). Because of some of these properties, we have proposed that it is mainly used by cells as a short-range NO-derived signalling mechanism that can be favoured in proteins located at a short distance from the sources of NO, namely NOS, and in proteins interacting with them (Martínez-Ruiz *et al.*, 2013).

In this work, we review the role of S-nitrosation and other NO signalling pathways in neuronal plasticity. The S-nitrosated proteins discussed in this review are summarized in Table 1.

S-nitrosation in synaptic plasticity

Synaptic plasticity refers essentially to the modification of the synaptic response upon intense or decreased synaptic input at a given synapse, resulting in long-lasting potentiation or depression of the synaptic function (see Ho *et al.*, 2011b; Choquet and Triller, 2013). Both presynaptic and postsynaptic changes in synaptic function can occur, but for the purpose of this review, we will focus on postsynaptic alterations of synaptic strength. The presynaptic modulation of synaptic plasticity by NO has been the focus of a recent comprehensive review (Hardingham *et al.*, 2013) and will not be discussed here. The changes in synaptic strength can be achieved by different mechanisms in different synapses throughout the brain. In the hippocampus, for instance, glutamate release and glutamate receptors are generally associated with LTP, and LTP is achieved by altering the number of AMPA receptors on the postsynaptic membrane. More receptors activated each time glutamate is released means a sustained larger excitatory postsynaptic potential (EPSP), while the opposite (fewer AMPA receptors on the post synaptic membrane) holds true for LTD. LTP is usually expressed by a larger amplitude in EPSP following a high-frequency stimulation, while LTD is characterized by a decrease in the amplitude of EPSP after low-frequency stimulation. LTP can be dependent on NMDA receptors, which are fundamental for modifying synaptic strength in the initial stages of LTP, as they trigger the signalling pathways that result in the mobilization of more AMPA receptors towards the postsynaptic membrane (Bliss and Collingridge, 2013). NMDA-independent mechanisms for LTP are also characterized (Oren *et al.*, 2009), but will not be discussed in this work. The mechanism for LTD induction may involve also NMDA

Table 1

Proteins modified by S-nitrosation that contribute to brain plasticity

Protein	S-nitrosated Cys	Functional significance	Experimental context	References
NSF	Cys[91]	Increase of membrane insertion of AMPA receptors GluA2 subunits	NO donors increase NSF-GluA2 association; impairment of GluA2 surface expression in nNOS-deficient mice	Huang et al., 2005
Stargazin	Cys[3]	Up-regulation of AMPA receptors in the nucleus accumbens	NO donors elicit S-nitrosation of stargazin	Selvakumar et al., 2009
GluA1	Cys[875]	Enhancement of Ser[831] phosphorylation, facilitation of the associated increase in AMPA receptor conductance and endocytosis	GluA1 S-nitrosation in cells overexpressing WT GluA1, but not GluA1-C875S, following exposure to NO donors or in HEK-nNOS cells	Selvakumar et al., 2013
GluN2A	Cys[399]	Down-regulation of NMDA receptor activity and decrease of calcium influx	Exogenous and endogenous NO-related species elicit S-nitrosation of NR2A in vitro	Choi et al., 2000; Kim et al., 1999
GluN1	Cys[744]/Cys[798]	Regulation of calcium influx through the NMDA receptor	S-nitrosation of GluN1 in cerebrocortical cultures and nNOS-HEK cells under hypoxic conditions	Takahashi et al., 2007
PSD-95	Cys[3]/Cys[5]	Reduction of synaptic clustering in granule cells of the cerebellum	S-nitrosation following exposure to exogenous or endogenous NO, and in WT mice, but not in nNOS^{−/−} mice	Ho et al., 2011a
Serine racemase	Cys[113]	Inhibition of enzyme activity and feedback mechanism to decrease the presynaptic formation of D-serine	S-nitrosation and enzyme inhibition using NO donors in vitro	Mustafa et al., 2007
RyR1	Cys[3635]	Increase of calcium release from the endoplasmic reticulum	S-nitrosation of RyR1 following exposure to a NO donor	Kakizawa et al., 2012
HDAC2	Cys[262]/Cys[274]	Chromatin remodelling, dendritic growth and branching, and the activation of genes associated with cortical development, regulating radial migration of cortical neurons	Endogenous NO production by NOS, but not in nNOS^{−/−} animals, and NO donors induces S-nitrosation of HDAC2	Nott et al., 2013; Nott et al., 2008
Cdk5	Cys[83]/Cys[157]	Contribution to NMDA-induced dendritic spine loss and neuronal apoptosis caused by amyloid-β damage	NO donors elicit Cdk5 activation; S-nitrosation of Cdk5 in human brain with Alzheimer's disease	Qu et al., 2011
Drp1	Cys[644]	Dendritic spine loss and neurodegeneration in Alzheimer's disease	NO donors and endogenous nNOS-derived NO induces Drp1 S-nitrosation	Cho et al., 2009
EGF receptor	Cys[166]/Cys[305]	Negative regulation of NSC proliferation in the SVZ by down-regulation of the ERK/MAPK pathway	EGF receptor is S-nitrosated in vitro in the presence of NO donors	Murillo-Carretero et al., 2009
H-Ras	Cys[118]	Alteration of the active centre conformation, allowing a faster transition from GDP- to GTP-binding state	NO donors activate WT H-Ras, but not H-Ras NO-insensitive mutant	Lander et al., 1997; Lander et al., 1995

receptors in some brain regions, such as the hippocampus, while metabotropic glutamate receptors are involved in LTD induction in the cerebellum, for instance. Thus, the mechanism for LTP or LTD is region- and synapse-specific. For the purpose of this review, we will focus on NMDA receptor-dependent potentiation of synaptic activity.

Physiologically, the activation of NMDA receptors results in calcium influx through the receptor channel. To induce LTP, calcium will activate PKs, such as calcium/calmodulin-dependent PK II (CaMKII), which will phosphorylate AMPA receptors present in postsynaptic pools of vesicles, making

them insert into the postsynaptic membrane (see Ho et al., 2011b). Phosphorylation of AMPA receptors by CaMKII and other kinases will also increase channel conductance and opening probability (Lee et al., 2000; 2010). The calcium influx via NMDA receptors that triggers LTP is also coupled to the postsynaptic synthesis of NO, given the close proximity of nNOS with NMDA receptors because of the interaction with the scaffolding postsynaptic density protein 95 (PSD-95) (Sattler et al., 1999). The small distance between nNOS and the NMDA receptor allows the production of NO when calcium enters the postsynaptic neuron directly through the

NMDA receptor, while calcium entering the postsynaptic neuron by other channels does not necessarily increase NO production (Sattler et al., 1999). The importance of NO for LTP triggered by activation of NMDA receptors was demonstrated by several laboratories in the early 1990s, showing that LTP was abolished when neurons were treated with NOS inhibitors (Böhme et al., 1991; 1993; O'Dell et al., 1991; Mizutani et al., 1993; Zhuo et al., 1993). The membrane-impermeable scavenger of NO, haemoglobin, also blocks LTP when applied extracellularly, suggesting that NO must be released extracellularly to the synaptic cleft for the occurrence of LTP (O'Dell et al., 1991). Furthermore, mice lacking only nNOS (O'Dell et al., 1994) or eNOS (Dinerman et al., 1994) are capable of normal LTP, but, in the presence of NOS inhibitors, LTP is reduced, suggesting that NO is important for LTP in these knockout mice. Moreover, an impairment of hippocampal LTP was observed in double-knockout mice for nNOS and eNOS, and no further reduction of LTP was found following the use of NOS inhibitors, demonstrating that NO derived from both NOS is involved in LTP (Son et al., 1996). However, in mature neurons of the neocortex of adult animals, synaptic potentiation was reduced (over 42%) in mice lacking nNOS (Dachtler et al., 2011), and LTP was completely abolished in double-knockout mice for nNOS and the glutamate receptor subunit GluA1, but not in eNOS/GluA1 double-knockout mice. These findings suggest that nNOS-derived NO and GluA1 subunit of the AMPA receptor are required for experience-dependent plasticity in the neocortex (Dachtler et al., 2011), without any contribution by eNOS. The contribution of NO to LTP may also be through cGMP production and PKG activation (Zhuo et al., 1994; Gallo and Iadecola, 2011), but recent studies have shown that several intracellular proteins involved in LTP and in AMPA receptor trafficking can be S-nitrosated (see Tegeder et al., 2011; Martínez-Ruiz et al., 2013), including NMDA receptor subunits and proteins associated with AMPA receptor membrane insertion and internalization.

AMPA receptor trafficking to the postsynaptic membrane is fine-tuned by a variety of regulator and adaptor proteins. Synaptic plasticity is mostly driven by GluA1/GluA2-containing AMPA receptors being inserted into the membrane after high-frequency stimulation, while after synaptic strengthening a new equilibrium is achieved by the insertion of GluA2/GluA3 receptors (Shi et al., 2001). Membrane insertion of GluA1/GluA2 subunits is controlled by association with N-ethyl-maleimide sensitive factor (NSF), and S-nitrosation of Cys^{91} on the NSF protein was shown to drive the surface expression of AMPA receptors containing GluA2 subunits (Huang et al., 2005), by allowing the dissociation of protein interacting with C α-kinase 1 from AMPA receptors and favouring membrane expression (Sossa et al., 2007). The surface expression of GluA1-containing AMPA receptors is regulated by stargazin, which is physiologically S-nitrosated on Cys^3 (Selvakumar et al., 2009). Recently, S-nitrosation of stargazin was also shown to mediate the up-regulation of AMPA receptors in the nucleus accumbens shell of cocaine-sensitized rats during withdrawal (Selvakumar et al., 2014), which implicates S-nitrosation of stargazin in the pathophysiology of addiction mechanisms that involve up-regulation of membrane expression of AMPA receptors.

The GluA1 subunit itself is also a target for S-nitrosation. Cys^{875} may be modified following NO production triggered by NMDA receptor activation by glutamate and thus enhance phosphorylation of Ser^{831}, resulting in an increase in channel conductance (Selvakumar et al., 2013). In the same study, nitrosation of Cys^{875} was also shown to facilitate binding of AP2 protein and promote endocytosis of AMPA receptors (Selvakumar et al., 2013).

S-nitrosation was also observed in NMDA receptor GluN2A and GluN1 subunits, with implications for the activity and permeability of NMDA receptors and, thus, on postsynaptic calcium influx. NMDA receptor GluN2 subunits are coupled to nNOS by the PSD-95/Dlg/ZO-1 (PDZ) domains of PSD-95, at the postsynaptic density (Christopherson et al., 1999) and nitrosation of GluN2A Cys^{399} down-regulates NMDA receptor activity and decreases calcium influx (Kim et al., 1999; Choi et al., 2000). Moreover, following hypoxia or stroke, GluN1 subunits appear nitrosated at the Cys^{744}/Cys^{798} pair, regulating calcium influx through the NMDA receptor, decreasing neurotoxicity (Takahashi et al., 2007). PSD-95 may also be regulated by S-nitrosation on Cys^3/Cys^5 (leading to reduced synaptic clustering); S-palmitoylation of the same residues favours synaptic localization of PSD-95 (Ho et al., 2011a). Furthermore, serine racemase, which converts L-serine to D-serine, was shown to be influenced by NO. D-Serine works as a co-agonist of NMDA receptors. Thus, in the presence of NO, serine racemase is S-nitrosated at Cys^{113} and its enzymatic activity is inhibited, creating a feedback mechanism to decrease the presynaptic formation of D-serine (Mustafa et al., 2007) after activation of glutamate receptors, resulting in decreased signalling by the NMDA receptor.

In the cerebellum, burst stimulation of the parallel fibres triggers NO-dependent LTP in the parallel fibre-Purkinje cell synapse (Namiki et al., 2005). NO was shown to increase release of calcium from the endoplasmic reticulum via S-nitrosation of Cys^{3635} in type 1 ryanodine receptors (RyR1), thus triggering the calcium increase necessary for increasing surface expression of AMPA receptors on the postsynaptic membrane in Purkinje cells (Kakizawa et al., 2012).

In dorsal root ganglion (DRG) neurons, which are important in nociception, NO inhibited the different types of sodium channels via S-nitrosation of C-type DRG neurons and consequently abolished sodium currents (Renganathan et al., 2002). The blockage of sodium channels conductance by a cGMP-independent mechanism could modulate the excitability of these neurons and contribute to impaired impulse in pathological conditions (Renganathan et al., 2002). Moreover, NO was also shown to activate ATP-sensitive potassium channels in large DRG neurons via S-nitrosation of cysteine residues in the SUR1 subunit (Kawano et al., 2009). Thus, by increasing the levels of NO, the currents of the ATP-sensitive potassium channels could be selectively enhanced even following a decrease in current caused by painful-like nerve injury (Kawano et al., 2009).

Overall, S-nitrosation of proteins involved in synaptic plasticity contributes to physiological receptor trafficking and synapse activity, and also affords neuroprotection in injury conditions. Recent evidence also indicates that S-nitrosation may be involved in sensitization to some drugs of abuse such as cocaine.

S-nitrosation and formation of new synapses

Synaptogenesis is the process that involves the modification and elimination of existing synapses and the formation of new synapses, resulting in the enhancement of learning and memory formation, and can be modulated by NO. Despite the relatively little information regarding its role in synaptic rearrangement in adulthood, NO is mainly described as a positive modulator of developmental synaptogenesis (Roskams et al., 1994; Sunico et al., 2005). NO is required for the refinement of retinal axonal connections (Wu et al., 1994) and for the formation and maintenance of neuronal projections in the developing and regenerating olfactory system (Roskams et al., 1994). Moreover, an increase in NOS expression seems to follow the axonal regenerative phase (Roskams et al., 1994). In the hippocampus, the postsynaptic scaffolding protein PSD-95 interacts with NOS and induces NO signalling, promoting synapse formation with nearby axons during development (Nikonenko et al., 2008). Another study with the NOS inhibitor Nω-nitro-L-arginine methyl ester demonstrated the relevance of NO to the formation of synaptic connections, as NOS inhibition decreased the immunoreactivity of synapsin I and NADPH-diaphorase activity in the prefrontal cortex, hippocampus and dorsal thalamus (Sánchez-Islas and León-Olea, 2004). The close association between nNOS, the nNOS adapter protein, C-terminal PDZ domain ligand of nNOS, and synapsins (proteins associated with presynaptic membrane vesicles and important for the regulation of several functions including synaptic plasticity, neurotransmitter release, synaptogenesis and neuronal proliferation) seems to be responsible for this effect, and NO deficiency affects learning and memory as a result of impairment in synaptic connections (Sánchez-Islas and León-Olea, 2004).

More recently, some studies report the effect of NO on synaptogenesis during adulthood. An increase in the immunoreactivity of synaptophysin was observed in the hippocampal dentate gyrus, 72 h following epileptic seizures, suggesting increased levels of synaptogenesis in this region. Treatment with the selective nNOS inhibitor Nω-propyl-L-arginine suppresses the increase in synaptogenesis, suggesting that nNOS plays an important role in seizure generation and in injury-induced synaptogenesis (Beamer et al., 2012). Indeed, studies with in vivo models of epileptic seizures showed a high rate of post-injury synaptic recovery in the hippocampus (Nadler et al., 1980; Phelps et al., 1991). Moreover, in hippocampal slices, following anoxia/hypoglycaemia stimuli, the postsynaptic NMDA receptors are activated, thus increasing the calcium-induced release of NO, which stimulates growth of presynaptic filopodia-like protrusions and remodelling of presynaptic varicosities, contributing to synaptogenesis (Nikonenko et al., 2003). On the other hand, there are several reports showing a negative effect of NO on motoneuron synaptogenesis (Sunico et al., 2005; 2010; Moreno-López et al., 2011). During adulthood, NO produced following injury to motoneuron axons is anti-synaptotrophic as it leads to synapse detachment by cGMP-dependent mechanisms, and is also anti-synaptogenic because of the inhibition of synapse formation through a S-nitrosation-mediated mechanism (Sunico et al., 2005). Indeed, a recovery of synaptic coverage was observed following nNOS inhibition, suggesting that neuronal NO blocks the CNS restorative events by a cGMP-dependent mechanism (Sunico et al., 2005). Moreover, NO from nNOS inhibits the formation of synapses necessary to recover normal functionality after injury through an increase in S-nitrosation of proteins in the synaptic bouton-like structures. However, the protein targets of S-nitrosation remain to be identified (Sunico et al., 2005).

Neurotrophins such as brain-derived neurotrophic factor (BDNF) have a pivotal role in the differentiation, cell survival and synapse formation in the CNS. Increasing BDNF levels enhances axon arborization and synapse formation during development, thus reversing the effect of NO, and suggesting that the anti-synaptotrophic and anti-synaptogenic functions of NO manifested following an injury might be due to abnormal levels of neurotrophic factors (Alsina et al., 2001). In neurons, BDNF triggers the synthesis of NO and subsequent S-nitrosation of two cysteine residues (Cys^{262} and Cys^{274}) of histone deacetylase 2 (HDAC2) in embryonic cortical neurons. In turn, S-nitrosated HDAC2 promotes chromatin remodelling, dendritic growth and branching, and the activation of genes associated with development (Nott et al., 2008).

Regarding the involvement of S-nitrosation in synaptic remodelling in neurodegenerative disorders, evidence of cyclin-dependent kinase 5 (Cdk5) being a target for the effect of NO in spine remodelling was found. S-nitrosation of Cys^{83} and Cys^{157} promote the activation of Cdk5, and this event was further demonstrated to contribute to dendritic spine loss caused by amyloid-β damage (Qu et al., 2011). Dynamin-related protein 1 (Drp1) can also be S-nitrosated in Cys^{644} and this modification has been linked to dendritic spine loss and neurodegeneration in Alzheimer's disease (Cho et al., 2009), although phosphorylation of Drp1 in Ser^{616} has been described in another study as a more likely modification that promotes mitochondrial dysfunction and synaptic loss (Bossy et al., 2010). Other proteins have been described to be involved in neurodegenerative processes and this topic has been recently reviewed by others (García-García et al., 2012; Halloran et al., 2013; Nakamura et al., 2013).

More information is needed concerning the proteins involved in synapse formation that are S-nitrosated, but the relevance of this PTM is already becoming clear. The identification of more proteins susceptible of being S-nitrosated during the synaptogenic event as well as identifying the cysteines modified is critical. As several neurodegenerative diseases also affect the de novo formation of synapses, targeting the critical residues could be of therapeutic interest in limiting the progression and promoting the recovery of neurons and neuronal function in these pathologies.

S-nitrosation and neurogenesis

Neurogenesis is the process that involves the formation of new neural cells from neural stem cells (NSC). Neurogenesis occurs primarily during embryogenesis, when self-renewing NSC are multipotent and give rise to the different neuroec-

todermal lineages of the CNS (Gage *et al.*, 1995). Nowadays, it is commonly accepted that neurogenesis also occurs throughout adult life. In the adult brain, there are two main neurogenic niches where NSC reside and can be recruited to give rise to new neurons: the subventricular zone in the wall of the lateral ventricles and the subgranular zone in the dentate gyrus of the hippocampus (see Gage and Temple, 2013). During adulthood, constitutive neurogenesis is involved in neural turnover in the olfactory bulb, and in learning and memory in the hippocampus respectively. The neurogenic process comprises several steps including proliferation, migration, survival and integration of the newly formed neurons into the neuronal networks of the CNS. Moreover, following certain conditions, such as neurodegenerative diseases and acute brain injuries, neurogenesis is impaired and modulation of endogenous neurogenesis could be a strategy to overcome these pathologies (Whitney *et al.*, 2009).

NO has been reported as a regulator of neurogenesis (see Carreira *et al.*, 2012). The role of NO in neurogenesis has been described as neurogenic or antineurogenic in several studies. Although some studies using NO donors and/or transgenic animals describe NO as an antineurogenic molecule (Packer *et al.*, 2003; Moreno-López *et al.*, 2004; Zhu *et al.*, 2006; Torroglosa *et al.*, 2007), others show the potential of NO in increasing different steps of neurogenesis, including proliferation of NSC, migration and differentiation into new neuronal cells (Cheng *et al.*, 2003; Lu *et al.*, 2003; Zhu *et al.*, 2003; Contestabile and Ciani, 2004; Covacu *et al.*, 2006; Luo *et al.*, 2007; Carreira *et al.*, 2010; 2013; Tegenge *et al.*, 2010). Furthermore, the amount and the source of NO are determining factors for this dual role of NO. While NO derived from nNOS has an inhibitory effect (Park *et al.*, 2002; Sun *et al.*, 2005; Fritzen *et al.*, 2007), NO produced by iNOS in response to an injury, such as repeated seizures, ischemia or traumatic brain injury, increases the proliferation rate of NSC and the migration and differentiation into new neuronal cells (Reif *et al.*, 2004; Luo *et al.*, 2007; Carreira *et al.*, 2010).

Although most of the effects of NO in NSC proliferation are mediated by the activation of ERK/MAPK and sGC/cGMP/PKG pathways (Carreira *et al.*, 2013), protein S-nitrosation appears to be important for the activation and/or inhibition of several proteins involved in the neurogenic process. Several proteins susceptible to S-nitrosation are involved in the control of cell proliferation. In the subventricular zone, the EGF receptor is S-nitrosated following treatment with NO donors and, thus, negatively regulates proliferation of NSC by down-regulation of the ERK/MAPK pathway. Moreover, a study with NO-insensitive mutants identified Cys^{166} and Cys^{305} of the EGF receptor as the targets of S-nitrosation (Murillo-Carretero *et al.*, 2009). A more recent study reported that NO was able to stimulate NSC proliferation by the activation of ERK/MAPK pathway, an effect that bypasses the EGF receptor, suggesting that NO acts downstream of this receptor in the modulation of cell proliferation by this pathway; moreover, Ras, an element downstream of the EGF receptor, appears likely to be the first target of NO (Carreira *et al.*, 2010). It has been described that NO triggers the activation of H-Ras through S-nitrosation of Cys^{118}, positioned near the active centre; this modification alters the active centre conformation, allowing a faster transition from GDP- to GTP-binding state (Lander *et al.*, 1995; 1997). It remains to be established whether S-nitrosation of Ras is directly correlated to the up-regulation of neurogenesis by NO.

Some studies have attributed the down-regulation of cAMP response element-binding protein (CREB) transcriptional activity to the action of NO produced by nNOS, which has an antiproliferative effect, both in the hippocampal dentate gyrus and in the subventricular zone (Packer *et al.*, 2003; Moreno-López *et al.*, 2004). CREB is activated by phosphorylation following an extracellular signal such as neurotrophins or neurotransmitters. CREB activity (but not CREB itself) seems to be modulated by a NO-dependent signalling pathway through S-nitrosation, as CREB activation is induced by BDNF and is dependent on S-nitrosation of components in the CREB DNA-binding complex. Thus, BDNF induces S-nitrosation of proteins associated with the *c-fos*, *nNOS* and *VGF* promoters and S-nitrosation of histone H3 (Riccio *et al.*, 2006), thus regulating CREB activity through S-nitrosation of some of its binding partners. However, the molecular pathway linking NO to down-regulation of CREB activity in neurogenesis remains to be characterized.

HDAC2 is involved in neurogenesis, and the catalytic function of HDAC2 was thought to be required only for adult neurogenesis, but not for embryonic neurogenesis (Jawerka *et al.*, 2010). However, during development of the cortex, S-nitrosation of HDAC2 at Cys^{262}/Cys^{274} was observed following the increase of NO production induced by BDNF, resulting in the regulation of the radial migration of neurons during cortical development (Nott *et al.*, 2013). S-nitrosation of regulators of neuronal development is part of the physiological signalling of embryogenesis.

As there is evidence showing that S-nitrosation is able to modify a number of transcription factors and regulate gene expression (see Sha and Marshall, 2012), it would be interesting to investigate if their modification could affect neurogenesis and brain development. The identification of the cysteine residues of proteins involved in the neurogenic process is essential for the better understanding of the functional role of the modifications.

Conclusion

NO has been widely recognized for its physiological functions at the CNS level, which can be both beneficial and detrimental. Pathophysiological levels of NO, similar to those found in neurodegenerative diseases, can be responsible for the progression of the disease by the S-nitrosation and other PTM of several proteins (see Nakamura *et al.*, 2013). Additionally, the identification of the proteins that are abnormally S-nitrosated in a particular neurodegenerative disorder will help to better target those proteins and, thus, address their function or signalling pathway in order to develop possible therapeutic approaches. In this regard, the constant development of more precise thiol redox proteomic techniques (Chouchani *et al.*, 2011; Izquierdo-Álvarez and Martínez-Ruiz, 2011; Martínez-Acedo *et al.*, 2012; López-Sánchez *et al.*, 2014) and their application to the field may be a valuable help in this task.

In physiological conditions, S-nitrosation is involved in the regulation of brain plasticity, as described in this review,

including synaptic plasticity and formation of new neurons and of new synapses. Here, we reviewed several proteins modified by S-nitrosation of specific Cys residues that contribute, directly or indirectly, to brain plasticity. Although some of these proteins may be possible therapeutic targets for increasing brain plasticity and, thus, ameliorate brain recovery following a lesion of a chronic neurodegenerative state, more studies are needed in order to better understand the implications of S-nitrosation in the pathogenesis and future therapeutic possibilities of neurodegenerative disorders.

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Author contributions

A. I. S., A. M-R. and I. M. A. wrote, reviewed and edited the manuscript.

Conflict of interest

The authors of this paper declare no conflict of interests.

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