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S-Nitrosylation in neurogenesis and neuronal development

Shu-ichi Okamoto* and Stuart A. Lipton*

Neuroscience and Aging Research Center, Sanford-Burnham Medical Research Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA

Department of Neurosciences, University of California San Diego, School of Medicine, 9500 Gilman Drive, La Jolla, CA 92048, USA

Abstract

Background—Nitric oxide (NO) is a pleiotropic messenger molecule. The multidimensional actions of NO species are, in part, mediated by their redox nature. Oxidative posttranslational modification of cysteine residues to regulate protein function, termed S-nitrosylation, constitutes a major form of redox-based signaling by NO.

Scope of Review—S-Nitrosylation directly modifies a number of cytoplasmic and nuclear proteins in neurons. S-Nitrosylation modulates neuronal development by reaction with specific proteins, including the transcription factor MEF2. This review focuses on the impact of S-nitrosylation on neurogenesis and neuronal development.

Major Conclusions—Functional characterization of S-nitrosylated proteins that regulate neuronal development represents a rapidly emerging field. Recent studies reveal that S-nitrosylation-mediated redox signaling plays an important role in several biological processes essential for neuronal differentiation and maturation.

General Significance—Investigation of S-nitrosylation in the nervous system has elucidated new molecular and cellular mechanisms for neuronal development. S-Nitrosylated proteins in signaling networks modulate key events in brain development. Dysregulation of this redox-signaling pathway may contribute to neurodevelopmental disabilities such as autism spectrum disorder (ASD). Thus, further elucidation of the involvement of S-nitrosylation in brain development may offer potential therapeutic avenues for neurodevelopmental disorders.

Keywords

Nitric oxide; Redox signaling; S-Nitrosylation; CREB; MEF2; Neurodevelopmental disorder

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*Corresponding authors. Tel.: +1 858 795 5260, sokamoto@sbmri.org (S.-i. Okamoto), slipton@ucsd.edu (S.A. Lipton).

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1. Redox signaling by S-nitrosylation

NO was first identified as an Endothelium Derived Relaxing Factor (EDRF) [1], but also serves as a more widespread signaling molecule [2]. NO-mediated physiological processes include vasodilation, immune function, neurotransmission, and neuronal maturation [2–6]. Three types of NO synthases generate endogenous NO in mammalian cells: neuronal NOS (nNOS, NOS1); inducible NOS (iNOS, NOS2); and endothelial NOS (eNOS, NOS3) [2, 7, 8]. All three NOS isoforms are expressed in the brain [9], but differential expression of the three isoforms with different characteristics orchestrates the diverse range of NO-mediated biochemical reactions [2]. However, excessive or sustained production of NO and NO-derived reactive nitrogen species (RNS) contributes to pathological consequences, including tissue damage, carcinomas, inflammatory conditions, and neurodegenerative diseases [3, 4]. Initial studies found that NO binds to the heme moiety of guanylate cyclase and induces its active conformation, resulting in the formation of cyclic GMP to induce vasodilation [2, 5]. Subsequently, studies elucidated that S-nitrosylation, an oxidative modification of cysteine thiol by NO-related species, affects protein activity and stability, protein-protein interactions, and protein trafficking and location. In many ways, S-nitrosylation serves as a posttranslational modification reminiscent of phosphorylation or acetylation, and, in fact, may be even more ubiquitous [2, 5]. The list of target proteins and physiological and pathological events evoked by S-nitrosylation is continually lengthening. S-Nitrosylation has emerged as a major mechanism for the signaling actions of NO.

Concerning the S-nitrosylation reaction, the d-orbitals of the sulfur atom at the thiol core confer high reactivity and chemical plasticity, allowing for multiple oxidation states (Fig. 1A), and are the main target of RNS for the formation of reversible S-nitrosothiols [5]. In some cases, e.g., matrix metalloproteinase-9 (MMP-9), S-nitrosylation subserves further irreversible oxidation, leading to sulfonation ($-\text{SO}_3\text{H}$) of the critical cysteine thiol [10]. S-Nitrosylation may be mediated, at least in part, by transnitrosylation reactions between proteins that transfer the NO group from the thiol of one protein to the thiol of another (Fig. 1B) [11–13]. In contrast, several denitrosylases have been reported to remove the NO group from S-nitrosylated thiol side chains [2, 14]. Thus, the balance between nitrosylation and denitrosylation tightly regulates the specific status of S-nitrosylation on cellular proteins. These regulatory mechanisms of S-nitrosylation offer control of many aspects of cellular physiology [2, 5]. Recent reviews have discussed the importance of S-nitrosylation on normal cellular physiology [5] as well as pathological states, particularly in the nervous system [2]. Here, we will focus on the role of S-nitrosylation in neurogenesis and neuronal development.

2. CREB (cyclic-AMP response element binding protein)-dependent dendritic growth and nitrosylation pathways

Dendrites are branched extensions from neurons that receive afferent inputs. The molecular and cellular mechanisms underlying dendritic development have been a focus of research for the past several decades. Recent studies revealed that dendritic development is regulated in both a neuronal activity-dependent and -independent manner [15, 16]. Both signaling pathways ultimately lead to activation of transcription factors such as the nuclear effector

CREB (cAMP response element-binding protein). CREB belongs to a family of transcription factors with a highly conserved basic region/leucine zipper domain. CREB was originally described as a cellular transcription factor that binds the cAMP-response element (CRE) [17]. CREB mediates calcium-dependent gene expression induced by depolarization in neuronal cells [18]. To date, CREB is arguably the best studied transcription factor in the context of the nervous system.

The activation of CREB is triggered by a wide variety of signaling processes in neurons. During dendritic growth, neuronal activity evokes calcium-dependent signaling and activates CREB kinases, causing phosphorylation of CREB serine-133, which results in CREB activation [19, 20]. In contrast, activity-independent signaling is elicited by extracellular factors such as neurotrophins. The neurotrophins encompass a family of closely related peptides, including brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), and NT-4 [21, 22]. Among them, BDNF is the most extensively studied molecule involved in dendritic development. BDNF induces NO generation and increases CREB binding activity in an nNOS-dependent and serine-133 phosphorylation independent manner [23, 24]. Interestingly, S-nitrosylation affects BDNF/CREB-dependent dendrite outgrowth at multiple levels of control, as described below [24–28].

One key action of BDNF concerns its effects on the localization of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and how this affects S-nitrosylation-mediated signaling. GAPDH is generally considered to be a housekeeping enzyme in the glycolysis cascade. However, GAPDH also acts as a signal mediator between the cytoplasm and the nucleus (despite its lack of a nuclear localization signal, NLS) [2, 29–31]. Importantly, the shuttling of GAPDH between the cytoplasm and nucleus is mediated by S-nitrosylation at the catalytic cysteine-150 (forming SNO-GAPDH) [25]. This S-nitrosylation reaction abolishes GAPDH enzymatic activity, but promotes its delivery to Siah (seven in absentia homolog 1, a ubiquitin E3 ligase) [25] (Fig. 2). Since Siah harbors an NLS, the SNO-GAPDH/Siah complex translocates to the nucleus [25]. Notably, this SNO-GAPDH translocation is negatively regulated by a second S-nitrosylation mechanism as follows. Sen et al. performed a yeast two-hybrid screen for proteins interacting with GAPDH and identified a cytoplasmic 52 kDa protein enriched in the brain, termed GOSPEL (GAPDH's competitor of Siah protein enhancer life) [26]. Under physiological conditions or low nitrosative stress, GOSPEL physically interacts with GAPDH in an S-nitrosylation-dependent manner. SNO-GOSPEL at cysteine-47 competes with Siah for binding to GAPDH, thereby retaining GAPDH in the cytoplasm [26] (Fig. 2).

The nuclear SNO-GAPDH/Siah complex enhances CREB-mediated dendrite outgrowth through at least two epigenetic chromatin-remodeling regulatory mechanisms. In the nucleus, SNO-GAPDH stabilizes Siah, which facilitates ubiquitination and degradation of nuclear proteins such as SUV39H1 [28]. SUV39H1 is a major histone methylating enzyme that trimethylates lysine 9 on histone H3 (H3K9), a molecular signature of gene silencing. In neurons, BDNF treatment induces the nuclear translocation of SNO-GAPDH/Siah and ubiquitin-mediated degradation of SUV39H1. This decrease in histone H3K9 trimethylation allows enhanced CREB binding [28] (Fig. 2). In addition, SNO-GAPDH propagates NO

signaling in the nucleus by transnitrosylation [27]. Since the NO group is transferred from the donor's SNO-thiol to the acceptor's thiol, donor proteins are regarded as transnitrosylases and acceptor proteins as denitrosylases (Fig. 1B). Recent studies have demonstrated transnitrosylation reactions in multiple biological systems [2, 14], e.g. SNO-hemoglobin and anion exchanger 1 [11], SNO-thioredoxin and caspase-3 [12, 32], SNO-caspase-3 and X-linked inhibitor of apoptosis, and SNO-cyclin-dependent kinase 5 and dynamin related protein 1 [33].

Nuclear translocated SNO-GAPDH can also serve as a transnitrosylase [27]. SNO-GAPDH interacts with nuclear proteins, including sirtuin-1, DNA-activated protein kinase, B23, and histone deacetylase 2 (HDAC2), in an S-nitrosylation dependent fashion [27, 34]. The transnitrosylation reaction can confer selectivity by targeted transfer of an NO group to reactive cysteine residues of specific substrates [27]. In the case of HDAC2, it is S-nitrosylated from SNO-GAPDH by transnitrosylation, and this reaction is also involved in BDNF-induced dendritic outgrowth [24] (Fig. 2). Under basal conditions, HDAC2 is associated with promoters of CREB target genes. BDNF stimulation induces S-nitrosylation of cysteine-262 and -274 on HDAC2 causing dissociation from these promoters. The dissociation induces rapid acetylation of histones H3 and H4, and expression of CREB target genes, leading to dendritic growth. These findings show that nitrosylation of HDAC2 promotes epigenetic changes and activation of CREB-dependent genes that are essential for dendritic development [24] (Fig. 2). Taken together, these experiments suggest that diverse S-nitrosylation signaling pathways convey extracellular cues to the nucleus and control the activity of two key chromatin modifiers (SUV39H1 and HDAC2) to influence dendritic outgrowth. Importantly, chromatin remodeling or epigenetic alteration acts as an interface between genetic risk and environmental factors, and, as such, is an evolving biological mechanism for gene x environment (GxE) interactions in the pathogenesis of an array of neurodevelopmental disorders, including schizophrenia and autism [35–38]. Any disturbance in the epigenetic remodeling cascade of SNO-GAPDH/Siah/HDAC2-SUV39H1/CREB by an environmental factor that causes excessive NO generation may potentially contribute to the pathology of neurodevelopmental disorders. Future work in this area may well yield new pathogenic insight into these disorders.

2. Microtubule-associated protein 1B (MAP1B) and S-nitrosylation in axonal retraction

An axon is the neuronal process that serves to relay afferent signals via action potential propagation. Axonal guidance, outgrowth, and retraction are coordinated by dynamic rearrangements of the actin and microtubule cytoskeletons. The coordinated remodeling of the cytoskeleton is essential for brain development. Missense and splice site mutations in α - and β -tubulin isotypes, constituents of neuronal microtubules, cause human neurodevelopmental disorders such as lissencephaly [39]. Additionally, the quality control or proteostasis of actin and tubulin proteins is strictly regulated during neuronal maturation in the developing brain. Abnormal expression or mutation in cytoskeletal proteostasis genes causes a spectrum of neurological disorders [40]. Notably, one key regulator of the dynamics of actin filaments and microtubules involves NO signaling. NO is known to

contribute to the refinement of axonal projections. For example, axons from retinal ganglion cells synapse onto neurons in the lateral geniculate nucleus of the thalamus in a highly-ordered manner. Visual function depends on proper axonal connections. Inappropriate projections are eliminated during visual system development. NO promotes axonal retraction during this connection refinement [41–43].

Microtubule-associated proteins (MAPs), a group of filamentous proteins, control the integrity and dynamics of microtubules. MAP1B is highly expressed in the developing brain [44] and, along with tau, is the main member of the neuronal MAPs. MAP1B protein undergoes proteolytic cleavage to generate MAP1B heavy chain (HC) and light chain (LC1), and forms a protein complex (HC/LC1) [44]. MAP1B is an essential regulator of the axonal cytoskeleton [44], controlling assembly and stability of both actin filaments and microtubules [45]. Studies using MAP1B knockout mice elucidated that MAP1B is necessary for axonal elongation and guidance *in vivo* [46, 47]. In addition, dysregulation of MAP1B has been implicated in the pathogenesis of neurodevelopmental disorders, including fragile X syndrome [48], spinocerebellar ataxia type 1 [49], and giant axonal neuropathy [50]. MAP1B has been reported to mediate nNOS-dependent axon retraction [51]. nNOS physically interacts with LC1, but not HC, and cysteine-2457 on LC1 is S-nitrosylated (Fig. 3A). This S-nitrosylation reaction changes the conformation of LC1 and results in increased binding of the HC/LC1 MAP1B complex to microtubules. This leads to axonal retraction, possibly by inhibiting the action of dynein, which is necessary for axonal extension [51].

3. S-Nitrosylated myocyte enhancer factor 2 (MEF2) in adult neurogenesis

Active neurogenesis continues throughout life in the adult brain of mammals, including humans [52, 53]. Adult neurogenesis is not observed throughout the brain, however, but is mainly restricted to two distinct areas: the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus (DG) [52, 53]. A recent study assessed the presence of nuclear bomb-test derived ^{14}C in genomic DNA and calculated that approximately 700 neurons are added every day with an annual turnover rate of 1.75% in the human DG [54]. The newly-generated neurons differentiate into granule neurons and integrate into the existing hippocampal circuitry, contributing to hippocampus-dependent learning and memory [52, 53]. Accumulating evidence shows that these new neurons play a pivotal role in fear conditioning [55], spatial and object recognition memory [56], and pattern separation [57]. Notably, adult neurogenesis in the DG is affected in psychiatric and neurological disorders, temporal lobe epilepsy [58], depression [59], bipolar disorder [60, 61], schizophrenia [61–63], Huntington's disease [54], and Alzheimer's disease (AD) [64].

MEF2 is a member of the MADS (MCM1, Agamous, Deficiens, and Serum response factor) box superfamily of transcription factors [65]. Yeast and invertebrates such as *Drosophila* and *Caenorhabditis elegans* possess a single MEF2, while there are four isoforms, MEF2A, B, C, and D, in vertebrates [65]. Our group originally cloned MEF2C and found it in the developing human brain [66]. The four MEF2 members are expressed in differential but overlapping expression patterns, in both the temporal and spatial domains, in developing and adult tissues. In general, MEF2 expression is abundant in muscle, lymphocytes, and neurons

[65]. The N-terminus consists of the MADS-box and MEF2 domains, which are highly conserved across species, and facilitate dimerization and DNA binding [65].

We and others have shown that MEF2 is involved in many different aspects of brain function, from embryonic development to neuronal survival and synaptic plasticity. A neuronal function of MEF2 that was recognized early on involves its pro-survival activity [67, 68]. Excitotoxic insults or environmental toxins inactivate MEF2, contributing to neuronal cell death [69–71]. MEF2 also controls synapse formation and dendritic remodeling, which is associated with neuronal plasticity, memory and learning. In hippocampal neurons, MEF2 modulates dendritic spine numbers in a neuronal activity-dependent manner [72, 73]. Sumoylation and acetylation of MEF2A regulates morphogenesis of dendritic claws in cerebellar neurons [74]. In addition, MEF2 drives embryonic neurogenesis [68, 70, 75]. Intriguingly, our group and that of Eric Olson (University Texas Southwestern) reported that *Mef2c*-specific knockout mice in the brain can result in autistic phenotypes and defects in learning and memory [73, 75]. Following those reports, *MEF2C* microdeletions, duplications, and mutations have been increasingly identified in human patients with autism spectrum disorder (ASD) and intellectual disability [65].

Importantly, MEF2 also promotes adult neurogenesis [76, 77]. Adult hippocampal neurogenesis is extremely plastic. A number of environmental or extrinsic factors have been shown to regulate hippocampal neurogenesis [52, 78]. Multiple studies using nNOS knockout mice or systemic or intraventricular administration of NOS inhibitors in rodents demonstrated that NO negatively regulates adult neurogenesis [79–81]. Interestingly, we found that MEF2A is the major MEF2 isoform in adult hippocampal progenitor cells [77], and MEF2 can be S-nitrosylated at cysteine-39 in the MADS/DNA binding domain. S-Nitrosylation of MEF2 (forming SNO-MEF2) reduces its binding to DNA and, thus, its transcriptional activity (Fig. 3B). Impacting neurogenesis, formation of SNO-MEF2 diminishes the expression of nuclear receptor tailless (TLX) [77], which is an essential regulator of adult neurogenesis and adult neurogenesis-dependent learning and memory [82–84]. Comparing the effects of wild-type MEF2 to a non-nitrosylatable MEF2 mutant, we inferred that SNO-MEF2 impairs neuronal differentiation from adult hippocampal progenitors (Fig. 3B). In disease states, we found evidence for aberrant formation of high levels of SNO-MEF2 in the brain, particularly in transgenic AD model mice and in human AD patients. Moreover, expression of non-nitrosylatable MEF2 increased adult hippocampal neurogenesis in the DG of transgenic AD model mice, consistent with the notion that SNO-MEF2 contributes to the pathological alteration of adult neurogenesis in AD [77]. As an ancillary question, whether formation of SNO-MEF2 affects embryonic neurogenesis is still under investigation. Along these lines, since mutations in the *MEF2C* gene cause ASD and intellectual disabilities [65], the potential involvement of an environmental toxin that could S-nitrosylate and thus inactivate MEF2C (as has been reported for pesticide-induced SNO-MEF2C in Parkinson's disease [71]), would be expected to mimic the effect of loss-of-function MEF2 mutations that result in neurodevelopmental disorders.

4. Perspectives for the future

Emerging evidence suggests the significance of redox-signaling networks, in large measure mediated by S-nitrosylation. Here, we discuss the importance of these reactions in neurogenesis and neuronal development. Analysis of individual proteins has revealed that S-nitrosylation reactions regulate the activity (either inhibition or activation) of specific proteins; alternatively, S-nitrosylation can affect the binding of one protein to another in a complex. For example, the SNO-GAPDH/SNO-HDAC2 cascade promotes dendritic outgrowth (Fig. 2), whereas formation of SNO-MAP1B LC1 causes retraction of axons (Fig. 3A). Moreover, generation of SNO-MEF2 inhibits neurogenesis (Fig. 3B). Thus, redox networks of S-nitrosylated proteins may fine-tune neuronal differentiation, development, and maturation in the developing brain.

As a caveat to present work, functional validation of the effects of S-nitrosylation of each protein has often relied on overexpression of a mutant protein – and this can introduce artifacts into the analysis. For example, non-physiological levels of mutant proteins might not recapitulate the action of physiological levels of endogenous proteins and can also be mislocalized in the cell. In contradistinction, new methods allow the endogenous gene to be mutated, thus preserving the endogenous protein level but introducing a desired mutation. For example, the CRISPR/Cas9 system has rapidly become a useful tool for genome editing in mammalian cells [85]. This genome editing approach is fast and efficient [85], and CRISPR/Cas9 tools have been used to generate genetically-engineered mice [85]. Specifically, this approach allows the endogenous gene, and hence its protein product, to be mutated to remove a specific nitrosylation site. To further confirm the role of S-nitrosylation in neurogenesis and neuronal development both *in vitro* and *in vivo*, these genome editing approaches will be essential. Furthermore, for *in vivo* analysis, the genomic conversion of a nitrosylatable site to a non-nitrosylatable site is a straightforward approach. For example, nNOS knockout mice manifest defects in dendritic branching [86], delays in axon degeneration (Wallerian degeneration) [87], and decreased adult neurogenesis [79], potentially because of a lack of S-nitrosylation of specific proteins. Using CRISPR/Cas9, it will be important to investigate if mutation of these nitrosylation sites can correct the neurodevelopmental phenotypes observed in nNOS knockout mice. These *in vivo* approaches will help elucidate the functional relevance of S-nitrosylation of specific targets during brain development.

Additionally, in the last several years, mass spectrometry-based methodologies have been developed to detect S-nitrosylated proteins and S-nitrosylation sites [88–90]. By applying proteomics to the investigation of neurogenesis and neuronal development, a more comprehensive view of S-nitrosylated proteins (the “S-nitrosoproteome”) should become available and provide further insight into the role of a number of S-nitrosylated proteins in brain development. These studies should provide a more targeted approach to uncover new S-nitrosylation pathways or networks crucial for neurogenesis and neuronal development. Importantly, since nitrosative stress is associated with neurodevelopmental disorders such as ASD [91, 92], exploration of S-nitrosylation pathways may lead to identification of molecular mechanisms for these diseases, and this may facilitate further treatment strategies.

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References

1. Martin W, Villani GM, Jothianandan D, Furchgott RF. Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. *J Pharmacol Exp Ther*. 1985; 232:708–716. [PubMed: 2983068]
2. Nakamura T, Tu S, Akhtar MW, Sunico CR, Okamoto S-i, Lipton SA. Aberrant protein S-nitrosylation in neurodegenerative diseases. *Neuron*. 2013; 78:596–614. [PubMed: 23719160]
3. Foster MW, McMahon TJ, Stamler JS. S-Nitrosylation in health and disease. *Trends Mol Med*. 2003; 9:160–168. [PubMed: 12727142]
4. Foster MW, Hess DT, Stamler JS. Protein S-nitrosylation in health and disease: a current perspective. *Trends Mol Med*. 2009; 15:391–404. [PubMed: 19726230]
5. Hess DT, Matsumoto A, Kim SO, Marshall HE, Stamler JS. Protein S-nitrosylation: purview and parameters. *Nat Rev Mol Cell Biol*. 2005; 6:150–166. [PubMed: 15688001]
6. Sen N, Snyder SH. Protein modifications involved in neurotransmitter and gasotransmitter signaling. *Trends Neurosci*. 2010; 33:493–502. [PubMed: 20843563]
7. Förstermann U, Schmidt HH, Pollock JS, Sheng H, Mitchell JA, Warner TD, Nakane M, Murad F. Isoforms of nitric oxide synthase. Characterization and purification from different cell types. *Biochem Pharmacol*. 1991; 42:1849–1857. [PubMed: 1720618]
8. Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. *Biochem J*. 2001; 357:593–615. [PubMed: 11463332]
9. Park CS, Krishna G, Ahn MS, Kang JH, Chung WG, Kim DJ, Hwang HK, Lee JN, Paik SG, Cha YN. Differential and constitutive expression of neuronal, inducible, and endothelial nitric oxide synthase mRNAs and proteins in pathologically normal human tissues. *Nitric Oxide*. 2000; 4:459–471. [PubMed: 11020335]
10. Gu Z, Kaul M, Yan B, Kridel SJ, Cui J, Strongin A, Smith JW, Liddington RC, Lipton SA. S-Nitrosylation of matrix metalloproteinases: signaling pathway to neuronal cell death. *Science*. 2002; 297:1186–1190. [PubMed: 12183632]
11. Pawloski JR, Hess DT, Stamler JS. Export by red blood cells of nitric oxide bioactivity. *Nature*. 2001; 409:622–626. [PubMed: 11214321]
12. Mitchell DA, Marletta MA. Thioredoxin catalyzes the S-nitrosation of the caspase-3 active site cysteine. *Nat Chem Biol*. 2005; 1:154–158. [PubMed: 16408020]
13. Nakamura T, Wang L, Wong CC, Scott FL, Eckelman BP, Han X, Tzitzilonis C, Meng F, Gu Z, Holland EA, Clemente AT, Okamoto S-i, Salvesen GS, Riek R, Yates JR 3rd, Lipton SA. Transnitrosylation of XIAP regulates caspase-dependent neuronal cell death. *Mol Cell*. 2010; 39:184–195. [PubMed: 20670888]
14. Benhar M, Forrester MT, Stamler JS. Protein denitrosylation: enzymatic mechanisms and cellular functions. *Nat Rev Mol Cell Biol*. 2009; 10:721–732. [PubMed: 19738628]
15. Redmond L, Ghosh A. Regulation of dendritic development by calcium signaling. *Cell Calcium*. 2005; 37:411–416. [PubMed: 15820388]
16. Bengtson CP, Bading H. Nuclear calcium signaling. *Adv Exp Med Biol*. 2012; 970:377–405. [PubMed: 22351065]
17. Montminy MR, Bilezikjian LM. Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. *Nature*. 1987; 328:175–178. [PubMed: 2885756]
18. Sheng M, McFadden G, Greenberg ME. Membrane depolarization and calcium induce c-fos transcription via phosphorylation of transcription factor CREB. *Neuron*. 1990; 4:571–582. [PubMed: 2157471]

19. West AE, Chen WG, Dalva MB, Dolmetsch RE, Kornhauser JM, Shaywitz AJ, Takasu MA, Tao X, Greenberg ME. Calcium regulation of neuronal gene expression. *Proc Natl Acad Sci USA*. 2001; 98:11024–11031. [PubMed: 11572963]
20. Hong EJ, West AE, Greenberg ME. Transcriptional control of cognitive development. *Curr Opin Neurobiol*. 2005; 15:21–28. [PubMed: 15721740]
21. Huang EJ, Reichardt LF. Trk receptors: roles in neuronal signal transduction. *Annu Rev Biochem*. 2003; 72:609–642. [PubMed: 12676795]
22. Mattson MP. Glutamate and neurotrophic factors in neuronal plasticity and disease. *Ann NY Acad Sci*. 2008; 1144:97–112. [PubMed: 19076369]
23. Riccio A, Alvania RS, Lonze BE, Ramanan N, Kim T, Huang Y, Dawson TM, Snyder SH, Ginty DD. A nitric oxide signaling pathway controls CREB-mediated gene expression in neurons. *Mol Cell*. 2006; 21:283–294. [PubMed: 16427017]
24. Nott A, Watson PM, Robinson JD, Crepaldi L, Riccio A. S-Nitrosylation of histone deacetylase 2 induces chromatin remodelling in neurons. *Nature*. 2008; 455:411–415. [PubMed: 18754010]
25. Hara MR, Agrawal N, Kim SF, Cascio MB, Fujimuro M, Ozeki Y, Takahashi M, Cheah JH, Tankou SK, Hester LD, Ferris CD, Hayward SD, Snyder SH, Sawa A. S-Nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. *Nat Cell Biol*. 2005; 7:665–674. [PubMed: 15951807]
26. Sen N, Hara MR, Ahmad AS, Cascio MB, Kamiya A, Ehmsen JT, Agrawal N, Hester L, Dore S, Snyder SH, Sawa A. GOSPEL: a neuroprotective protein that binds to GAPDH upon S-nitrosylation. *Neuron*. 2009; 63:81–91. [PubMed: 19607794]
27. Kornberg MD, Sen N, Hara MR, Juluri KR, Nguyen JV, Snowman AM, Law L, Hester LD, Snyder SH. GAPDH mediates nitrosylation of nuclear proteins. *Nat Cell Biol*. 2010; 12:1094–1100. [PubMed: 20972425]
28. Sen N, Snyder SH. Neurotrophin-mediated degradation of histone methyltransferase by S-nitrosylation cascade regulates neuronal differentiation. *Proc Natl Acad Sci USA*. 2011; 108:20178–20183. [PubMed: 22123949]
29. Sirover MA. New insights into an old protein: the functional diversity of mammalian glyceraldehyde-3-phosphate dehydrogenase. *Biochim Biophys Acta*. 1999; 1432:159–184. [PubMed: 10407139]
30. Sawa A, Khan AA, Hester LD, Snyder SH. Glyceraldehyde-3-phosphate dehydrogenase: nuclear translocation participates in neuronal and nonneuronal cell death. *Proc Natl Acad Sci USA*. 1997; 94:11669–11674. [PubMed: 9326668]
31. Shahani N, Sawa A. Protein S-nitrosylation: role for nitric oxide signaling in neuronal death. *Biochim Biophys Acta*. 2012; 1820:736–742.
32. Benhar M, Forrester MT, Hess DT, Stamler JS. Regulated protein denitrosylation by cytosolic and mitochondrial thioredoxins. *Science*. 2008; 320:1050–1054. [PubMed: 18497292]
33. Qu J, Nakamura T, Cao G, Holland EA, McKercher SR, Lipton SA. S-Nitrosylation activates Cdk5 and contributes to synaptic spine loss induced by beta-amyloid peptide. *Proc Natl Acad Sci USA*. 2011; 108:14330–14335. [PubMed: 21844361]
34. Lee SB, Kim CK, Lee KH, Ahn JY. S-Nitrosylation of B23/nucleophosmin by GAPDH protects cells from the SIAH1-GAPDH death cascade. *J Cell Biol*. 2012; 199:65–76. [PubMed: 23027902]
35. Labrie V, Pai S, Petronis A. Epigenetics of major psychosis: progress, problems and perspectives. *Trends Genet*. 2012; 28:427–435. [PubMed: 22622229]
36. Diwadkar VA, Bustamante A, Rai H, Uddin M. Epigenetics, stress, and their potential impact on brain network function: a focus on the schizophrenia diatheses. *Front Psychiatry*. 2014; 5:71. [PubMed: 25002852]
37. Jeste SS, Geschwind DH. Disentangling the heterogeneity of autism spectrum disorder through genetic findings. *Nat Rev Neurol*. 2014; 10:74–81. [PubMed: 24468882]
38. Tordjman S, Somogyi E, Coulon N, Kermarrec S, Cohen D, Bronsard G, Bonnot O, Weismann-Arcache C, Botbol M, Lauth B, Ginchat V, Roubertoux P, Barburroth M, Kovess V, Geoffroy MM, Xavier J. Gene x Environment interactions in autism spectrum disorders: role of epigenetic mechanisms. *Front Psychiatry*. 2014; 5:53. [PubMed: 25136320]

39. Tischfield MA, Cederquist GY, Gupta ML Jr, Engle EC. Phenotypic spectrum of the tubulin-related disorders and functional implications of disease-causing mutations. *Curr Opin Genet Dev.* 2011; 21:286–294. [PubMed: 21292473]
40. Lundin VF, Leroux MR, Stirling PC. Quality control of cytoskeletal proteins and human disease. *Trends Biochem Sci.* 2010; 35:288–297. [PubMed: 20116259]
41. Wu HH, Williams CV, McLoon SC. Involvement of nitric oxide in the elimination of a transient retinotectal projection in development. *Science.* 1994; 265:1593–1596. [PubMed: 7521541]
42. Cramer KS, Angelucci A, Hahm JO, Bogdanov MB, Sur M. A role for nitric oxide in the development of the ferret retinogeniculate projection. *J Neurosci.* 1996; 16:7995–8004. [PubMed: 8987826]
43. Ernst AF, Wu HH, El-Fakahany EE, McLoon SC. NMDA receptor-mediated refinement of a transient retinotectal projection during development requires nitric oxide. *J Neurosci.* 1999; 19:229–235. [PubMed: 9870953]
44. Gordon-Weeks PR, Fischer I. MAP1B expression and microtubule stability in growing and regenerating axons. *Microsc Res Tech.* 2000; 48:63–74. [PubMed: 10649507]
45. Togel M, Wiche G, Propst F. Novel features of the light chain of microtubule-associated protein MAP1B: microtubule stabilization, self interaction, actin filament binding, and regulation by the heavy chain. *J Cell Biol.* 1998; 143:695–707. [PubMed: 9813091]
46. Meixner A, Haverkamp S, Wassele H, Fuhrer S, Thalhammer J, Kropf N, Bittner RE, Lassmann H, Wiche G, Propst F. MAP1B is required for axon guidance and is involved in the development of the central and peripheral nervous system. *J Cell Biol.* 2000; 151:1169–1178. [PubMed: 11121433]
47. Takei Y, Teng J, Harada A, Hirokawa N. Defects in axonal elongation and neuronal migration in mice with disrupted tau and MAP1b genes. *Journal Cell Biol.* 2000; 150:989–1000. [PubMed: 10973990]
48. Zhang YQ, Bailey AM, Matthies HJ, Renden RB, Smith MA, Speese SD, Rubin GM, Broadie K. *Drosophila* fragile X-related gene regulates the MAP1B homolog Futsch to control synaptic structure and function. *Cell.* 2001; 107:591–603. [PubMed: 11733059]
49. Opal P, Garcia JJ, Propst F, Matilla A, Orr HT, Zoghbi HY. Mapmodulin/leucine-rich acidic nuclear protein binds the light chain of microtubule-associated protein 1B and modulates neurogenesis. *J Biol Chem.* 2003; 278:34691–34699. [PubMed: 12807913]
50. Allen E, Ding J, Wang W, Pramanik S, Chou J, Yau V, Yang Y. Gigaxonin-controlled degradation of MAP1B light chain is critical to neuronal survival. *Nature.* 2005; 438:224–228. [PubMed: 16227972]
51. Stroissnigg H, Trancikova A, Descovich L, Fuhrmann J, Kutschera W, Kostan J, Meixner A, Nothias F, Propst F. S-nitrosylation of microtubule-associated protein 1B mediates nitric-oxide-induced axon retraction. *Nat Cell Biol.* 2007; 9:1035–1045. [PubMed: 17704770]
52. Faigle R, Song H. Signaling mechanisms regulating adult neural stem cells and neurogenesis. *Biochim Biophys Acta.* 2013; 1830:2435–2448. [PubMed: 22982587]
53. Yu DX, Marchetto MC, Gage FH. How to make a hippocampal dentate gyrus granule neuron. *Development.* 2014; 141:2366–2375. [PubMed: 24917496]
54. Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, Bostrom E, Westerlund I, Vial C, Buchholz BA, Possnert G, Mash DC, Druid H, Frisen J. Dynamics of hippocampal neurogenesis in adult humans. *Cell.* 2013; 153:1219–1227. [PubMed: 23746839]
55. Saxe MD, Battaglia F, Wang JW, Malleret G, David DJ, Monckton JE, Garcia AD, Sofroniew MV, Kandel ER, Santarelli L, Hen R, Drew MR. Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. *Proc Natl Acad Sci USA.* 2006; 103:17501–17506. [PubMed: 17088541]
56. Jessberger S, Clark RE, Broadbent NJ, Clemenson GD Jr, Consiglio A, Lie DC, Squire LR, Gage FH. Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learn Mem.* 2009; 16:147–154. [PubMed: 19181621]
57. Sahay A, Scobie KN, Hill AS, O'Carroll CM, Kheirbek MA, Burghardt NS, Fenton AA, Dranovsky A, Hen R. Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature.* 2011; 472:466–470. [PubMed: 21460835]

58. Hattiangady B, Shetty AK. Decreased neuronal differentiation of newly generated cells underlies reduced hippocampal neurogenesis in chronic temporal lobe epilepsy. *Hippocampus*. 2010; 20:97–112. [PubMed: 19309040]
59. Patricio P, Mateus-Pinheiro A, Sousa N, Pinto L. Re-cycling paradigms: cell cycle regulation in adult hippocampal neurogenesis and implications for depression. *Mol Neurobiol*. 2013; 48:84–96. [PubMed: 23471746]
60. Valvezan AJ, Klein PS. GSK-3 and Wnt Signaling in Neurogenesis and Bipolar Disorder. *Front Mol Neurosci*. 2012; 5:1. [PubMed: 22319467]
61. Walton NM, Zhou Y, Kogan JH, Shin R, Webster M, Gross AK, Heusner CL, Chen Q, Miyake S, Tajinda K, Tamura K, Miyakawa T, Matsumoto M. Detection of an immature dentate gyrus feature in human schizophrenia/bipolar patients. *Transl Psychiatry*. 2012; 2:e135. [PubMed: 22781168]
62. Tamminga CA, Stan AD, Wagner AD. The hippocampal formation in schizophrenia. *Am J Psychiatry*. 2010; 167:1178–1193. [PubMed: 20810471]
63. Hagihara H, Takao K, Walton NM, Matsumoto M, Miyakawa T. Immature dentate gyrus: an endophenotype of neuropsychiatric disorders. *Neural Plast*. 2013; 2013:318596. [PubMed: 23840971]
64. Mu Y, Gage FH. Adult hippocampal neurogenesis and its role in Alzheimer's disease. *Mol Neurodegener*. 2011; 6:85. [PubMed: 22192775]
65. Dietrich JB. The MEF2 family and the brain: from molecules to memory. *Cell Tissue Res*. 2013; 352:179–190. [PubMed: 23397426]
66. Leifer D, Krainc D, Yu YT, McDermott J, Breitbart RE, Heng J, Neve RL, Kosofsky B, Nadal-Ginard B, Lipton SA. MEF2C, a MADS/MEF2-family transcription factor expressed in a laminar distribution in cerebral cortex. *Proc Natl Acad Sci USA*. 1993; 90:1546–1550. [PubMed: 7679508]
67. Mao Z, Bonni A, Xia F, Nadal-Vicens M, Greenberg ME. Neuronal activity-dependent cell survival mediated by transcription factor MEF2. *Science*. 1999; 286:785–790. [PubMed: 10531066]
68. Okamoto, S-i; Krainc, D.; Sherman, K.; Lipton, SA. Antiapoptotic role of the p38 mitogen-activated protein kinase-myocyte enhancer factor 2 transcription factor pathway during neuronal differentiation. *Proc Natl Acad Sci USA*. 2000; 97:7561–7566. [PubMed: 10852968]
69. Okamoto, S-i; Li, Z.; Ju, C.; Schölzke, MN.; Mathews, E.; Cui, J.; Salvesen, GS.; Bossy-Wetzel, E.; Lipton, SA. Dominant-interfering forms of MEF2 generated by caspase cleavage contribute to NMDA-induced neuronal apoptosis. *Proc Natl Acad Sci USA*. 2002; 99:3974–3979. [PubMed: 11904443]
70. Li Z, McKercher SR, Cui J, Nie Z, Soussou W, Roberts AJ, Sallmen T, Lipton JH, Talantova M, Okamoto S-i, Lipton SA. Myocyte enhancer factor 2C as a neurogenic and antiapoptotic transcription factor in murine embryonic stem cells. *J Neurosci*. 2008; 28:6557–6568. [PubMed: 18579729]
71. Ryan SD, Dolatabadi N, Chan SF, Zhang X, Akhtar MW, Parker J, Soldner F, Sunico CR, Nagar S, Talantova M, Lee B, Lopez K, Nutter A, Shan B, Molokanova E, Zhang Y, Han X, Nakamura T, Masliah E, Yates JR 3rd, Nakanishi N, Andreyev AY, Okamoto S-i, Jaenisch R, Ambasudhan R, Lipton SA. Isogenic human iPSC Parkinson's model shows nitrosative stress-induced dysfunction in MEF2-PGC1alpha transcription. *Cell*. 2013; 155:1351–1364. [PubMed: 24290359]
72. Flavell SW, Cowan CW, Kim TK, Greer PL, Lin Y, Paradis S, Griffith EC, Hu LS, Chen C, Greenberg ME. Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number. *Science*. 2006; 311:1008–1012. [PubMed: 16484497]
73. Barbosa AC, Kim MS, Ertunc M, Adachi M, Nelson ED, McAnally J, Richardson JA, Kavalali ET, Monteggia LM, Bassel-Duby R, Olson EN. MEF2C, a transcription factor that facilitates learning and memory by negative regulation of synapse numbers and function. *Proc Natl Acad Sci USA*. 2008; 105:9391–9396. [PubMed: 18599438]
74. Shalizi A, Gaudilliere B, Yuan Z, Stegmuller J, Shirogane T, Ge Q, Tan Y, Schulman B, Harper JW, Bonni A. A calcium-regulated MEF2 sumoylation switch controls postsynaptic differentiation. *Science*. 2006; 311:1012–1017. [PubMed: 16484498]

75. Li H, Radford JC, Ragusa MJ, Shea KL, McKercher SR, Zaremba JD, Soussou W, Nie Z, Kang YJ, Nakanishi N, Okamoto S-i, Roberts AJ, Schwarz JJ, Lipton SA. Transcription factor MEF2C influences neural stem/progenitor cell differentiation and maturation in vivo. *Proc Natl Acad Sci USA*. 2008; 105:9397–9402. [PubMed: 18599437]
76. Petrik D, Jiang Y, Birnbaum SG, Powell CM, Kim MS, Hsieh J, Eisch AJ. Functional and mechanistic exploration of an adult neurogenesis-promoting small molecule. *FASEB J*. 2012; 26:3148–3162. [PubMed: 22542682]
77. Okamoto, S-i; Nakamura, T.; Cieplak, P.; Chan, SF.; Kalashnikova, E.; Liao, L.; Saleem, S.; Han, X.; Clemente, A.; Nutter, A.; Sances, S.; Brechtel, C.; Haus, D.; Haun, F.; Sanz-Blasco, S.; Huang, X.; Li, H.; Zaremba, JD.; Cui, J.; Gu, Z.; Nikzad, R.; Harrop, A.; McKercher, SR.; Godzik, A.; Yates, JR., 3rd; Lipton, SA. S-Nitrosylation-mediated redox transcriptional switch modulates neurogenesis and neuronal cell death. *Cell Rep*. 2014; 8:217–228. [PubMed: 25001280]
78. Benarroch EE. Adult neurogenesis in the dentate gyrus: general concepts and potential implications. *Neurology*. 2013; 81:1443–1452. [PubMed: 24078736]
79. Packer MA, Stasiv Y, Benraiss A, Chmielnicki E, Grinberg A, Westphal H, Goldman SA, Enikolopov G. Nitric oxide negatively regulates mammalian adult neurogenesis. *Proc Natl Acad Sci USA*. 2003; 100:9566–9571. [PubMed: 12886012]
80. Moreno-Lopez B, Romero-Grimaldi C, Noval JA, Murillo-Carretero M, Matarredona ER, Estrada C. Nitric oxide is a physiological inhibitor of neurogenesis in the adult mouse subventricular zone and olfactory bulb. *J Neurosci*. 2004; 24:85–95. [PubMed: 14715941]
81. Torroglosa A, Murillo-Carretero M, Romero-Grimaldi C, Matarredona ER, Campos-Caro A, Estrada C. Nitric oxide decreases subventricular zone stem cell proliferation by inhibition of epidermal growth factor receptor and phosphoinositide-3-kinase/Akt pathway. *Stem Cells*. 2007; 25:88–97. [PubMed: 16960136]
82. Liu HK, Belz T, Bock D, Takacs A, Wu H, Lichter P, Chai M, Schutz G. The nuclear receptor *tailless* is required for neurogenesis in the adult subventricular zone. *Genes Dev*. 2008; 22:2473–2478. [PubMed: 18794344]
83. Zhang CL, Zou Y, He W, Gage FH, Evans RM. A role for adult TLX-positive neural stem cells in learning and behaviour. *Nature*. 2008; 451:1004–1007. [PubMed: 18235445]
84. Murai K, Qu Q, Sun G, Ye P, Li W, Asuelime G, Sun E, Tsai GE, Shi Y. Nuclear receptor TLX stimulates hippocampal neurogenesis and enhances learning and memory in a transgenic mouse model. *Proc Natl Acad Sci USA*. 2014; 111:9115–9120. [PubMed: 24927526]
85. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. *Cell*. 2014; 157:1262–1278. [PubMed: 24906146]
86. Inglis FM, Furia F, Zuckerman KE, Strittmatter SM, Kalb RG. The role of nitric oxide and NMDA receptors in the development of motor neuron dendrites. *J Neurosci*. 1998; 18:10493–10501. [PubMed: 9852587]
87. Keilhoff G, Fansa H, Wolf G. Differences in peripheral nerve degeneration/regeneration between wild-type and neuronal nitric oxide synthase knockout mice. *J Neurosci Res*. 2002; 68:432–441. [PubMed: 11992469]
88. Seth D, Stamler JS. The SNO-proteome: causation and classifications. *Curr Opin Chem Biol*. 2011; 15:129–136. [PubMed: 21087893]
89. Foster MW. Methodologies for the characterization, identification and quantification of S-nitrosylated proteins. *Biochim Biophys Acta*. 2012; 1820:675–683. [PubMed: 21440604]
90. Raju K, Doulias PT, Tenopoulou M, Greene JL, Ischiropoulos H. Strategies and tools to explore protein S-nitrosylation. *Biochim Biophys Acta*. 2012; 1820:684–688. [PubMed: 21651963]
91. Frustaci A, Neri M, Cesario A, Adams JB, Domenici E, Dalla Bernardina B, Bonassi S. Oxidative stress-related biomarkers in autism: systematic review and meta-analyses. *Free Radic Biol Med*. 2012; 52:2128–2141. [PubMed: 22542447]
92. Colvin SM, Kwan KY. Dysregulated nitric oxide signaling as a candidate mechanism of fragile X syndrome and other neuropsychiatric disorders. *Front Genet*. 2014; 5:239. [PubMed: 25101118]

Highlights

- S-Nitrosylation networks modulate neuronal differentiation and development
- S-Nitrosylated GAPDH/Siah/CREB cascade controls dendritic growth
- S-Nitrosylation of MAP1B regulates axonal retraction
- S-Nitrosylation of MEF2 serves as a negative switch for adult neurogenesis

Fig. 1.

Progressive posttranslational oxidation of cysteine thiols and mechanism of transnitrosylation. A) Reactive nitrogen species (RNS) and/or reactive oxygen species (ROS) oxidize redox sensitive-cysteine thiols. For some protein cysteine residues, as RNS/ROS levels increase, the oxidation status of the thiol may progress from S-nitrosothiol (–SNO), to sulfenic acid (–SOH) to sulfinic acid (–SO₂H), and finally to an irreversible sulfonic acid (–SO₃H) (as occurs for MMP-9) [10]. B) Transfer of a nitric oxide (NO) group from one protein to another (transnitrosylation) represents an enzymatic reaction mechanism for effecting nitrosation. The interaction of SNO-protein A with protein B determines the specificity of transnitrosylation. Since the NO group is transferred from SNO-protein A to a specific cysteine thiol on protein B, protein A is regarded as a nitrosylase and protein B as a denitrosylase (since protein A surrenders its NO group to protein B).

Fig. 2.

SNO-GAPDH/Siah/CREB machinery in BDNF-induced dendritic outgrowth. BDNF induces the generation of NO via nNOS and consequently S-nitrosylation of GAPDH (SNO-GAPDH) in the cytoplasm. SNO-GAPDH binds to Siah, and the complex then translocates into the nucleus. This process is negatively regulated by SNO-GOSPEL. Once in the nucleus, SNO-GAPDH stabilizes Siah, and Siah promotes degradation of SUV38H1 histone-methylating enzyme. In turn, SUV38H1 increases CREB binding to its target regulatory elements. Additionally, nuclear SNO-GAPDH transnitrosylates its NO group to HDAC2. The resulting SNO-HDAC2 manifests diminished DNA binding activity, which enhances CREB binding to target-gene promoters essential for dendritic outgrowth.

Fig. 3.

S-Nitrosylation-mediated regulation of axonal retraction and adult neurogenesis. A) NO enhances refinement of axonal processes during brain development. S-Nitrosylation of MAP1B light chain (forming SNO-LC1) promotes binding to microtubules of the MAP1B complex (consisting of heavy chain (HC) and LC1). This action increases axonal retraction. B) NO negatively regulates adult neurogenesis in the hippocampus. S-Nitrosylation of MEF2A (forming SNO-MEF2A) decreases the expression of TLX, a key nuclear receptor involved in adult neurogenesis. Thus, formation of SNO-MEF2 impairs adult neurogenesis, as demonstrated in recent *in vitro* and *in vivo* experiments [77].