REDOX PIONEER #10

Redox Pioneer: Professor Stuart A. Lipton

Jonathan S. Stamler

Professor Stuart A. Lipton

Abstract

Stuart A. Lipton, M.D., Ph.D. is recognized here as a Redox Pioneer because of his publication of four articles that have been cited more than 1000 times, and 96 reports which have been cited more than 100 times. In the redox field, Dr. Lipton is best known for his work on the regulation by S-nitrosylation of the NMDA-subtype of neuronal glutamate receptor, which provided early evidence for *in situ* regulation of protein activity by S-nitrosylation and a prototypic model of allosteric control by this post-translational modification. Over the past several years, Lipton's group has pioneered the discovery of aberrant protein nitrosylation that may contribute to a number of neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (Lou Gehrig's disease). In particular, the phenotypic effects of rare genetic mutations may be understood to be enhanced or mimicked by nitrosative (and oxidative) modifications of cysteines and thereby help explain common sporadic forms of disease. Thus, Lipton has contributed in a major way to the understanding that nitrosative stress may result from modifications of specific proteins and may operate in conjunction with genetic mutation to create disease phenotype. Lipton (collaborating with Jonathan S. Stamler) has also employed the concept of targeted S-nitrosylation to produce novel neuroprotective drugs that act at allosteric sites in the NMDA receptor. Lipton has won a number of awards, including the Ernst Jung Prize in Medicine, and is an elected fellow of the AAAS. *Antioxid. Redox Signal.* 19, 757–764.

The moment of insight into the solution of a scientific problem is what I find so exciting about analyzing experiments. It is humbling, however, that the study of post-translational redox modifications of proteins, such as S-nitrosylation, is perhaps 40 years behind that of phosphorylation—and our oxidized environment only makes it more arduous to approach empirically. Hence, cleverly designed, carefully controlled experiments are essential.

—Prof. Stuart A. Lipton

Education and Professional Training of Dr. Lipton

DR. LIPTON, BORN IN Connecticut, was recruited out of high school as a result of National Merit Finalist status to the Cornell University 6-year Ph.D. Program, an educational experiment funded by the Ford Foundation in which most students pursued careers in math or physics. Lipton was quickly attracted to biophysics and the relatively new Divi-

sion of Neurobiology and Behavior, where physics and chemistry were being used to help explain animal and (hopefully) human behavior. He performed research in neuroimmunology and electrophysiology. To pursue an idea he had formulated regarding the role of cyclic nucleotides in the mechanism of phototransduction, Lipton entered the NIH-sponsored Medical Scientist Training (M.D.-Ph.D.) Program at the University of Pennsylvania to work with

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Author note: Redox Pioneer Stuart Lipton provided initial evidence for S-nitrosylation of the NMDA receptor about 20 years ago. His subsequent work in this area has provided a paradigmatic model of nitrosative stress.

For a list of frequently cited articles published by Prof. Stuart A. Lipton, see Supplementary Tables S1 and S2, available online at www.liebertpub.com/ars

endocrinologist Howard Rasmussen, M.D., Ph.D. on cyclic nucleotides in rod photoreceptors and subsequently with Professor John Dowling in the Department of Biology at Harvard University, for electrophysiological analysis. He completed clinical training in internal medicine and neurology at Harvard at the Brigham and Women's/Beth Israel Deaconess/Children's Hospital program before continuing his postdoctoral research under Professor Torsten N. Wiesel, in the Department of Neurobiology at Harvard Medical School. Lipton remained at Harvard as a faculty member in the Department of Neurology for about 25 years before moving to La Jolla, California, 12 years ago as the founding director of a new Neuroscience, Aging, and Stem Cell Research Center at the Sanford-Burnham Medical Research Institute. In addition, he has enjoyed co-appointments as Professor at the Salk Institute for Biological Studies, the Scripps Research Institute, and the University of California, San Diego, where he is also an active clinical neurologist.

Summary of Dr. Lipton's Contributions to Redox Biology

Early studies (in collaboration with Jonathan S. Stamler) provided among the first *in situ* evidence that S-nitrosylation can regulate protein activity. More specifically, S-nitrosylation (elicited by NO group-donating agents) was shown to allosterically inhibit ligand-gated NMDA receptor channel activity, thus acting at the cell membrane to modulate signaling, in contrast to the prevailing view of NO as a freely diffusible and promiscuous gas (50, 55). The work would help set the stage for the current framework in which S-nitrosylation is recognized as exerting a very broad range of activities, akin to phosphorylation. Lipton's subsequent work showed that aberrant S-nitrosylation (in some cases followed by irreversible oxidation) may contribute to the pathogenesis of several neurological conditions. Aberrant S-nitrosylation is caused by nitrosative stress that accompanies aging, environmental toxin exposure, and disease (Fig. 1). As a clinical neurologist, Dr. Lipton would

FIG. 1. Mechanism through which S-nitrosylated species contribute to synaptic loss and neuronal cell death. Excessive activation of NMDA receptors triggers generation of NO and subsequent S-nitrosylation of neuronal proteins, contributing to synaptic damage and neuronal death. In contrast, disruption of either the NMDAR/PSD-95 complex or (PSD-95/nNOS interaction has been shown to decrease glutamate-induced neurotoxicity. Neuroinflammatory conditions, hypoxia/ischemia, pesticides, and soluble oligomers of amyloid beta (A β) can each contribute to a cascade of events, leading to increased NMDA receptor activity and thus facilitate NO production, resulting in a series of protein S-nitrosylation events that contribute to cellular injury. For example, in Parkinson's disease (PD), S-nitrosylation of parkin (SNO-parkin) disrupts its E3 ligase activity, thus mimicking rare genetic mutations of parkin that contribute to neuronal damage in PD. Parkin can also be further oxidized to sulfinic and sulfonic acid derivatives, which causes irreversible inhibition. In PD, Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS), the endoplasmic reticulum (ER) chaperone protein protein-disulfide isomerase (PDI) is S-nitrosylated, resulting in misfolding of multiple proteins. In AD and cerebral ischemia, the transcription factor MEF2C is S-nitrosylated to disrupt its neurogenic and anti-apoptotic activity. In cerebral ischemia, matrix metalloproteinases (MMPs)-2 and -9 are reversibly activated by S-nitrosylation, whereas subsequent oxidation to sulfinic acid and sulfonic acid results in irreversible activation. In human PD brains, peroxiredoxin 2 (Prx2) is S-nitrosylated to inhibit its antioxidant activity against peroxides, contributing to neuronal injury and death. In addition, in human PD brains, X-linked inhibitor of apoptosis (XIAP) is S-nitrosylated, potentially *via* transnitrosylation from caspases. S-Nitrosylation of XIAP inhibits its E3 ligase activity toward caspases, thereby stabilizing caspases and increasing cell death. In human AD and Huntington's disease (HD) brains, and possibly during the early stages of PD (at least in animal models), the mitochondrial fission protein dynamin-related protein 1 (Drp1) is S-nitrosylated, potentially *via* transnitrosylation from cyclin-dependent kinase 5 (Cdk5). SNO-Drp1 can contribute to synaptic damage and neuronal cell death by triggering excessive mitochondrial fission and bioenergetic impairment. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

champion the view that these findings have implications for the treatment of neurodegenerative diseases as well as HIV-associated neurocognitive disorders.

Description of Key Finding 1

Demonstration of the in situ regulation of neuronal protein function by S-nitrosylation and implications for neurodegenerative disease

Initially, S-nitrosylation of the NMDA receptor was described by Lipton and Stamler as a means to feedback inhibit the activity of this type of glutamate receptor (50), building on work by Lipton and colleagues (33) on the oxidation of the NMDA receptor redox modulatory site by NO donors (in which it was proposed that an S-nitrosothiol [SNO] may form en route to disulfide). These findings provided the basis for the idea that NO could have protective functions in the brain, to be contrasted with accepted dogma that implicated NO exclusively in neurotoxicity. Studies by Dr. Solomon Snyder, Dr. Joel Bockaert, and their coworkers would establish that these early studies with exogenous NO donors (33, 50), in fact, had important physiological and pathophysiological relevance (24, 59). Subsequent work by Lipton and coworkers identified the sites of S-nitrosylation in the NMDA receptor and indicated the operation of an oxygen-sensitive redox switch that primed S-nitrosylation (16, 31, 73, 78). In addition, Lipton and Stamler and colleagues would use the NMDA receptor as an exemplary case to help identify a motif comprising critical amino acids flanking the cysteines targeted by S-nitrosylation, which has subsequently been found in hundreds of proteins and may influence protein–protein interactions, subcellular location and activity. Lipton’s work also helped establish the relevance of protein–protein transnitrosylation in conveying SNO-based signals—that is, “nitrosylase” activities based on group-

transfer chemistry directly analogous to palmitoyl transferases, ubiquitin ligases, and acetylases—and he has demonstrated a role for aberrant nitrosylase activity in disease (61). These studies represent a continuation of work from the Lipton group that has implicated aberrant S-nitrosylation of multiple proteins in neurodegenerative disorders and stroke, including caspases, matrix metalloproteinases (MMPs), parkin, protein-disulfide isomerase (PDI), dynamin-related protein 1 (Drp1), X-linked inhibitor of apoptosis (XIAP), cyclin-dependent kinase 5 (Cdk5), peroxiredoxin 2 (Prx2), phosphatase and tensin homolog (PTEN), and myocyte enhancer factor 2 (MEF2) (15, 20, 23, 32, 53, 57, 61, 62, 67, 79, 82, 85).

Specific illustrations of Lipton’s contributions to our understanding of S-nitrosylation include:

- The demonstration that aberrant S-nitrosylation can mimic a rare genetic mutation to cause a neurodegenerative disease, with the discovery in Parkinson’s disease that parkin can be S-nitrosylated to ultimately decrease its E3 ubiquitin ligase activity (53, 85). This concept of genetic mimicry has been extended to Drp1, PDI, and MEF2, which may thereby contribute to Parkinson’s disease (parkin, PDI, and Drp1), Alzheimer’s disease (Drp1, PDI), amyotrophic lateral sclerosis (PDI), Huntington’s disease (Drp1), and autism (MEF2C). Genetic mimicry by aberrant nitrosylation may be helpful in explaining the neurotoxic effect of pesticides, fungicides, and herbicides, which have been linked to several of these conditions (60).
- The demonstration that nitrosative stress directly disrupts the protein-folding machinery of the endoplasmic reticulum (ER) *via* S-nitrosylation (and subsequent oxidation) of the enzyme PDI, thus potentially contributing

FIG. 2. Schematic of mechanism of SNO-XIAP-mediated neuronal cell death. (1) Usually, XIAP blocks caspases *via* interactions of BIR domains and also serves as an E3 ligase to ubiquitinate caspases for proteasomal degradation. (2) During nitrosative stress, NO inactivates XIAP E3 ligase activity *via* S-nitrosylation, thus preventing caspase degradation and sensitizing neurons to apoptotic stimuli. (3) S-Nitrosylated caspases also produce SNO-XIAP *via* transnitrosylation to promote neuronal cell death. Inasmuch as S-nitrosylation of caspases inhibits apoptotic activity, whereas S-nitrosylation of XIAP promotes apoptotic activity, the transfer of NO from caspase to XIAP serves as a molecular switch to activate apoptotic cascades [adapted from Nakamura *et al.* (61)]. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

to protein misfolding and aggregation in neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease (82).

- Demonstration of S-nitrosylation and consequent oxidation of MMP2/9 (to higher S-oxides), in pathological activation of these gelatinases, contributing to apoptotic cell death in cerebral ischemia (stroke) (23).
- Demonstration that synaptic damage in Alzheimer's disease may involve beta-amyloid peptide-mediated S-nitrosylation and activation of the mitochondrial fission protein, Drp1 (5, 15).
- Demonstration of protein-to protein transnitrosylation from caspase to XIAP (Fig. 2), which stimulates apoptotic signaling in the brain (61).

Description of Key Finding 2

Discovery of redox modulation of NMDA-type glutamate receptors, glycine receptors, and GABA_A receptors

Work from the laboratory of Arthur Karlin at Columbia had shown that the nicotinic subtype of acetylcholine receptor could be regulated by redox modulation. While studying this effect in retinal ganglion cell neurons, Elias Aizenman, then a postdoctoral fellow in the Lipton lab, along with collaborator Ralph Loring, discovered that the NMDA-type of glutamate receptor was also redox modulated (1). In collaboration with Steve Heinemann's laboratory, the Lipton group characterized these redox modulatory sites as comprising critical cysteine residues: A variety of thiol oxidants and reductants were shown to modulate the activity of NMDA receptors as well as other ion channel complexes, including GABA_A receptors and glycine receptors (15, 33–35, 51, 65, 75–77). The Lipton laboratory similarly showed that NO- and nitro-based compounds could alter channel activity through these redox-modulatory sites by producing oxidation (see Key Finding 1, above).

Description of Key Finding 3

Preclinical and clinical development of the NMDA-type glutamate receptor antagonist, memantine

Lipton has contributed to the understanding and development of the NMDA receptor antagonist Memantine (Name[®]nda) for the treatment of Alzheimer's disease. This work included characterization of the mechanism of action of memantine called "UFO" or Uncompetitive, Fast Off-rate action, which is proposed to account for its efficacy and clinical tolerability. Lipton also helped identify novel potential applications for memantine, and ran a successful clinical trial with the drug. Memantine represents the first clinically tolerated NMDA-type glutamate receptor antagonist to be approved by the FDA and the European Medicines Agency for clinical use and has been shown to limit both oxidative and nitrosative stress in neurons (11–14, 26, 40, 41, 43, 46–48, 54, 66, 74, 84, 87).

Other Achievements

- With colleagues Stamler, Wang, and Larrick, Lipton developed the allosteric drug NitroMemantine, which is designed to target NO specifically to NMDA receptors, and thereby to downregulate excessive receptor activity *via* active (memantine) and allosteric (S-nitrosylation) site regulation (47, 83).
- Demonstration that HIV coat protein gp120 causes neuronal damage and contributes to HIV-associated dementia *via* excessive activation of Ca²⁺ channels and NMDA receptor-activated channels (2, 10, 17–19, 21, 22, 25, 27–29, 36–40, 42–44, 52, 56, 63, 86). HIV-1 activates inducible NO synthase (iNOS or NOS2) in human microglia and contributes to neuronal cell injury (10).
- Characterization of apoptotic cell death pathways in neurodegenerative conditions, which are triggered by mild NMDA receptor overactivity and involve free

FIG. 3. Pro-electrophile to electrophile conversion by oxidative stress. During oxidative insult, pro-electrophiles are converted to electrophiles that can alkylate cysteine thiols. For example, a terpenoid with a catechol ring represents a pro-electrophile that becomes an electrophile: The catechol is converted to a quinone-type electrophile by oxidation. Subsequent nucleophilic attack of the electrophilic carbon (green area) modifies a specific cysteine residue in a target protein (R-SH, *e.g.*, Keap1-SH) to modulate protein function. In the case of the nuclear factor erythroid 2-related factor 2 (Nrf2)/Kelch-like ECH-associated protein 1 (Keap1) pathway, the reaction of the electrophile with Keap1 results in release of Nrf2 in the cytoplasm, subsequent entry of Nrf2 into the nucleus, and transcriptional activation of phase 2 anti-oxidant genes. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

radicals, in contrast to fulminant necrosis (3, 4, 7–9, 30, 45, 49, 58, 64, 80, 81).

- Characterization of the role of mitochondria in necrotic *versus* apoptotic neuronal cell death caused by excitotoxins and oxidants (3, 6, 9) (work performed in collaboration with Pierluigi Nicotera, DZNE Bonn).
- Cloning and characterization of a new family of NMDA receptor subunits, NR3A and NR3B (more recently designated GluN3A, GluN3B) (carried out in part in collaboration with Steve Heinemann, Salk Institute, San Diego, USA).
- Characterization of pro-electrophilic drugs (including the Rosemary herbal ingredient carnosic acid) as activators of the Kelch-like ECH-associated protein 1 (Keap1)/nuclear factor erythroid 2-related factor 2 (Nrf2) anti-oxidant pathway to afford neuroprotection. With collaborator Takumi Satoh in Japan, the Lipton laboratory showed that carnosic acid is an example of a catechol pro-drug that is converted by oxidation (and hence oxidizing environments) into a quinone derivative which reacts with critical cysteine residues in Keap1, releasing bound Nrf2 (Fig. 3). Nrf2 activates phase 2 anti-oxidant genes *via* the anti-oxidant redox element to confer neuroprotection (48, 68–72).

Conclusions

The current perspective on neurodegenerative conditions, including Parkinson's disease, Alzheimer's disease, and stroke, involves a critical role for nitrosative and oxidative stress that derives in significant part from Lipton's work. The common thread through this body of work is a focus on the NMDA subtype of glutamate receptor, which provides a model of NO/redox signaling and pathophysiology. Notable accomplishments include:

- Studies of the NMDA receptor, which helped establish the role of S-nitrosylation as an allosteric regulator of protein function.
- Pioneering studies demonstrating that aberrant S-nitrosylation may contribute to neurodegeneration.
- Identification of both nitrosative and oxidative modifications of proteins within a continuum of neuropathophysiology.
- Translation of these basic discoveries in redox-based regulation, including the development of novel drug therapies for neurodegenerative disease.

Short Quotation

That moment of insight into the solution of a scientific problem is what I find so exciting. However, then the empirical work begins, to prove whether the "insight" was correct or just a passing whim. Where is the enjoyment?—In reviewing the data, discussing it with colleagues, and making the next testable hypothesis. A humbling adage, however, should always be prescient in consciousness: As Karl Popper stated, "In so far as a scientific statement speaks about reality, it must be falsifiable," that is, we should be able to test it to show whether it is incorrect. In this light, the study of posttranslational redox modifications of proteins, such as S-nitrosylation, are perhaps 40 years behind that of phosphorylation—and our oxidized environment only makes it

more arduous to approach empirically. Hence, cleverly designed, carefully controlled experiments are essential.

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References

1. Aizenman E, Lipton SA, and Loring RH. Selective modulation of NMDA responses by reduction and oxidation. *Neuron* 2: 1257–1263, 1989.
2. Alirezaei M, Watry DD, Flynn CF, Kiosses WB, Masliah E, Williams BR, Kaul M, and Lipton SA. Human immunodeficiency virus-1/surface glycoprotein 120 induces apoptosis through RNA-activated protein kinase signaling in neurons. *J Neurosci* 27: 11047–11055, 2007.
3. Ankarcrona M, Dypbukt JM, Bonfoco E, Zhivotovsky B, Orrenius S, Lipton SA, and Nicotera P. Glutamate-induced neuronal death: A succession of necrosis or apoptosis depending on mitochondrial integrity. *Neuron* 15: 961–973, 1995.
4. Awobuluyi M, Yang J, Ye Y, Chatterton JE, Godzik A, Lipton SA, and Zhang D. Subunit-specific roles of glycine-binding domains in activation of NR1/NR3 N-methyl-D-aspartate receptors. *Mol Pharmacol* 71: 112–122, 2007.
5. Barsoum MJ, Yuan H, Gerencser AA, Liot G, Kushnareva Y, Gräber S, Kovacs I, Lee WD, Waggoner J, Cui J, White AD, Bossy B, Martinou J-C, Youle RJ, Lipton SA, Ellisman MH, Perkins GA, and Bossy-Wetzel E. Nitric oxide-induced mitochondrial fission is regulated by dynamin related GTPases in neurons. *EMBO J* 25: 3900–3911, 2006.
6. Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, and Lipton SA. Apoptosis and necrosis: two distinct events induced respectively by mild and intense insults with NMDA or nitric oxide/superoxide in cortical cell cultures. *Proc Natl Acad Sci U S A* 92: 7162–7166, 1995.

7. Bonfoco E, Leist M, Zhivotovsky B, Orrenius S, Lipton SA, and Nicotera P. Cytoskeletal breakdown and apoptosis elicited by NO-donors in cerebellar granule cells require NMDA-receptor activation. *J Neurochem* 67: 2484–2493, 1996.
8. Bossy-Wetzel E, Talantova MV, Lee WD, Schölkke MN, Harrop A, Mathews E, Götz T, Han J, Ellisman MH, Perkins GA, and Lipton SA. Cross-talk between nitric oxide and zinc pathways to neuronal cell death involving mitochondrial dysfunction and p38-activated K⁺ channels. *Neuron* 41: 351–365, 2004.
9. Budd SL, Tenneti L, Lishnak T, and Lipton SA. Mitochondrial and extramitochondrial apoptotic signaling pathways in cerebrocortical neurons. *Proc Natl Acad Sci U S A* 97: 6161–6166, 2000.
10. Bukrinsky MI, Nottet HSLM, Schmidtayerova H, Dubrovsky L, Flanagan CR, Mullins ME, Lipton SA, and Gendelman HE. Regulation of nitric oxide synthase activity in human immunodeficiency virus type 1 (HIV-1)-infected monocytes: Implications for HIV-associated neurological disease. *J Exp Med* 181: 735–745, 1995.
11. Chen H-SV and Lipton SA. Mechanism of memantine block of NMDA-activated channels in rat retinal ganglion cells: uncompetitive antagonism. *J Physiol (Lond)* 499: 27–46, 1997.
12. Chen H-SV and Lipton SA. Pharmacological implications of two distinct mechanisms of interaction of memantine with NMDA-gated channels. *J Pharmacol Exp Ther* 314: 961–971, 2005.
13. Chen H-SV, Pellegrini JW, Aggarwal SK, Lei SZ, Warach S, Jensen FE, and Lipton SA. Open-channel block of NMDA responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity. *J Neurosci* 12: 4427–4436, 1992.
14. Chen H-SV, Wang YF, Rayudu PY, Edgecomb P, Neill JC, Segal MM, Lipton SA, and Jensen FE. Neuroprotective concentrations of the NMDA open-channel blocker memantine are effective without cytoplasmic vacuolization following post-ischemic administration and do not block maze learning or LTP. *Neuroscience* 86: 1121–1132, 1998.
15. Cho D-H, Nakamura T, Fang J, Cieplak P, Godzik A, Gu Z, and Lipton SA. S-Nitrosylation of Drp1 mediates β -amyloid-related mitochondrial fission and neuronal injury. *Science* 302: 102–105, 2009.
16. Choi Y-B, Tenneti L, Le DA, Ortiz J, Bai G, Chen H-SV, and Lipton SA. Molecular basis of NMDA receptor-coupled ion channel modulation by S-nitrosylation. *Nat Neurosci* 3: 15–21, 2000.
17. Dreyer EB, Kaiser PK, Offermann JT, and Lipton SA. HIV-1 coat protein neurotoxicity prevented by calcium channel antagonists. *Science* 248: 364–367, 1990.
18. Dreyer EB and Lipton SA. The coat protein gp120 of HIV-1 inhibits astrocyte uptake of excitatory amino acids via macrophage arachidonic acid. *Eur J Neurosci* 7: 2502–2507, 1995.
19. Dreyer EB, Zurakowski D, Gorla M, Vorwerk CK, and Lipton SA. The contribution of various nitric oxide synthase gene products to HIV-1 coat protein (gp120)-mediated retinal ganglion cell injury. *Invest Ophthalmol Vis Sci* 40: 983–989, 1999.
20. Fang J, Nakamura T, Cho D-H, Gu Z, and Lipton SA. S-Nitrosylation of peroxiredoxin 2 (Prx2) promotes oxidative stress-induced neuronal cell death in Parkinson's disease. *Proc Natl Acad Sci U S A* 104: 18742–18747, 2007.
21. Garden G, Budd SL, Tsai E, Hanson L, Kaul M, D'Emilia DM, Friedlander RM, Yuan J, Masliah E, and Lipton SA. Caspase cascades in HIV-associated neurodegeneration. *J Neurosci* 22: 4015–4024, 2002.
22. Gelbard HA, Nottet HSLM, Swindells S, Jett M, Dzenko KA, Genis P, White R, Wang L, Choi Y-B, Zhang D, Lipton SA, Tourtellotte WW, Epstein LG, and Gendelman HE. Platelet-activating factor: A candidate human immunodeficiency virus type 1-induced neurotoxin. *J Virol* 68: 4628–4635, 1994.
23. Gu Z, Kaul M, Yan B, Kridel SJ, Cui J, Strongin A, Smith JW, Liddington RC, and Lipton SA. S-Nitrosylation of matrix metalloproteinases: signaling pathway to neuronal cell death. *Science* 297: 1186–1190, 2002.
24. Jaffrey SR, Erdjument-Bromage H, Ferris CD, Tempst P, and Snyder SH. Protein S-nitrosylation: a physiological signal for neuronal nitric oxide. *Nat Cell Biol* 3: 193–197, 2001.
25. Kaiser PK, Offermann JT, and Lipton SA. Neuronal injury due to HIV-1 envelope protein is blocked by anti-gp120 antibodies but not by anti-CD4 antibodies. *Neurology* 40: 1757–1761, 1990.
26. Karschin A, Aizenman E, and Lipton SA. The interaction of agonists and noncompetitive antagonists at the excitatory amino acid receptors in rat retinal ganglion cells *in vitro*. *J Neurosci Res* 8: 2895–2906, 1988.
27. Kaul M, Garden GA, and Lipton SA. Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature* 410: 988–994, 2001.
28. Kaul M and Lipton SA. Chemokines and activated macrophages in gp120-induced neuronal apoptosis. *Proc Natl Acad Sci U S A* 96: 8212–8216, 1999.
29. Kaul M, Qing M, Medders KE, and Lipton SA. HIV-1 coreceptors CCR5 and CXCR4 both mediate neuronal cell death but CCR5 paradoxically can also contribute to neuroprotection. *Cell Death Diff* 14: 296–305, 2007.
30. Kikuchi M, Tenneti L, and Lipton SA. Role of p38 mitogen-activated protein kinase in axotomy-induced apoptosis of rat retinal ganglion cells. *J Neurosci* 20: 5037–5044, 2000.
31. Kim W-K, Choi Y-B, Rayudu PV, Das P, Asaad W, Arnelle DR, Stamler JS, and Lipton SA. Attenuation of NMDA receptor activity and neurotoxicity by nitroxyl (NO⁻). *Neuron* 24: 461–469, 1999.
32. Kwak YD, Ma T, Diao S, Zhang X, Chen Y, Hsu J, Lipton SA, Masliah E, Xu H, and Liao FF. NO signaling and S-nitrosylation regulate PTEN inhibition in neurodegeneration. *Mol Neurodegener* 5: 49, 2010.
33. Lei SZ, Pan Z-H, Aggarwal SK, Chen H-SV, Hartman J, Sucher NJ, and Lipton SA. Effect of nitric oxide production on the redox modulatory site of the NMDA receptor-channel complex. *Neuron* 8: 1087–1099, 1992.
34. Levy DI, Sucher NJ, and Lipton SA. Redox modulation of NMDA receptor-mediated toxicity in mammalian central neurons. *Neurosci Lett* 110: 291–296, 1990.
35. Levy DI, Sucher NJ, and Lipton SA. Glutathione prevents NMDA receptor-mediated neurotoxicity. *NeuroReport* 2: 345–347, 1991.
36. Lipton SA. Calcium channel antagonists and human immunodeficiency virus coat protein-mediated neuronal injury. *Neuron* 30: 110–114, 1991.
37. Lipton SA. 7-Chlorokynurenate ameliorates neuronal injury mediated by HIV envelope protein gp120 in rodent retinal cultures. *Eur J Neurosci* 4: 1411–1415, 1992.
38. Lipton SA. Memantine prevents HIV coat protein-induced neuronal injury *in vitro*. *Neurology* 42: 1403–1405, 1992.
39. Lipton SA. Models of neuronal injury in AIDS: another role for the NMDA receptor?. *Trends Neurosci* 15: 75–79, 1992.

40. Lipton SA. Requirement for macrophages in neuronal injury induced by HIV envelope protein gp120. *NeuroReport* 3: 913–915, 1992.
41. Lipton SA. Human immunodeficiency virus-infected macrophages, gp120, and NMDA receptor-mediated neurotoxicity. *Ann Neurol* 33: 227–228, 1993.
42. Lipton SA. Prospects for clinically-tolerated NMDA antagonists: open-channel blockers and alternative redox states of nitric oxide. *Trends Neurosci* 16: 527–532, 1993.
43. Lipton SA. HIV coat protein gp120 induces soluble neurotoxins in culture medium. *Neurosci Res Commun* 15: 31–37, 1994.
44. Lipton SA. Neurobiology: HIV displays its coat of arms. *Nature* 367: 113–114, 1994.
45. Lipton SA. Janus Faces of NF- κ B: neurodestruction versus neuroprotection. *Nat Med* 3: 20–22, 1997.
46. Lipton SA. Concepts: turning down but not off—neuroprotection requires a paradigm shift in drug development. *Nature* 428: 473, 2004.
47. Lipton SA. Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. *Nat Rev Drug Disc* 5: 160–170, 2006.
48. Lipton SA. Pathologically activated therapeutics for neuroprotection. *Neurosci Lett* 8: 803–808, 2007.
49. Lipton SA. NMDA receptor activity regulates transcription of antioxidant pathways. *Nat Neurosci* 11: 381–382, 2008.
50. Lipton SA, Choi Y-B, Pan Z-H, Lei SZ, Chen H-SV, Sucher NJ, Singel DJ, Loscalzo J, and Stamler JS. A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. *Nature* 364: 626–632, 1993.
51. Lipton SA, Choi Y-B, Takahashi T, Zhang D, Li W, Godzik A, and Bankston LA. Cysteine regulation of protein function—as exemplified by NMDA-receptor modulation. *Trends Neurosci* 25: 474–480, 2002.
52. Lipton SA and Gendelman HE. Dementia associated with the acquired immunodeficiency syndrome. *N Engl J Med* 332: 934–940, 1995.
53. Lipton SA, Nakamura T, Uehara T, Shi Z-Q, and Gu Z. Comment on S-nitrosylation of parkin regulates ubiquitination and compromises parkin's protective function. *Science* 308: 1870, 2005.
54. Lipton SA and Rosenberg PA. Mechanisms of disease: excitatory amino acids as a final common pathway in neurologic disorders. *N Engl J Med* 330: 613–622, 1994.
55. Lipton SA, Stamler JS, and Singel DJ. NO comments. *Nature* 367: 28, 1994.
56. Lipton SA, Sucher NJ, Kaiser PK, and Dreyer EB. Synergistic effects of HIV coat protein and NMDA receptor-mediated neurotoxicity. *Neuron* 7: 111–118, 1991.
57. Manabe S, Gu Z, and Lipton SA. Activation of matrix metalloproteinase-9 via neuronal nitric oxide synthase contributes to NMDA-induced retinal ganglion cell death. *Invest Ophthalmol Vis Sci* 46: 4747–4753, 2005.
58. Manabe S and Lipton SA. NMDA-induced divergent signals leading to proapoptotic and antiapoptotic pathways in the rat retina. *Invest Ophthalmol Vis Neurosci* 44: 385–392, 2003.
59. Manzoni O and Bockaert J. Nitric oxide synthase activity endogenously modulates NMDA receptors. *J Neurochem* 61: 368–370, 1993.
60. Nakamura T, Tu S, Akhtar MW, Sunico CR, Okamoto S-i, and Lipton SA. Aberrant protein S-nitrosylation in neurodegenerative diseases. *Neuron* 78: 506–614, 2013.
61. Nakamura T, Wang L, Wong CCL, 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, and Lipton SA. Transnitrosylation of XIAP regulates caspase-dependent neuronal cell death. *Mol Cell* 39: 184–195, 2010.
62. Numajiri N, Takasawa K, Nishiya T, Hayakawa W, Asada M, Matsuda H, Azumi K, Tanaka H, Hyakkoku K, Kamata H, Nakamura T, Hara M, Lipton SA, and Uehara T. On-off system for PI3-kinase-Akt signaling through S-nitrosylation of phosphatase with sequence homology to tensin (PTEN). *Proc Natl Acad Sci U S A* 108: 10349–10354, 2011.
63. Okamoto S-i, Kand Y-J, Brechtel CW, Siviglia E, Russo R, Clemente A, Harrop A, McKercher S, Kaul M, and Lipton SA. HIV/gp120 decreases adult neural progenitor cell proliferation via checkpoint kinase-mediated cell-cycle withdrawal and G1 arrest. *Cell Stem Cell* 1: 230–236, 2007.
64. Okamoto S-i, Li Z, Ju C, Schölzke MN, Matthews E, Cui J, Salvesen GS, Bossy-Wetzel E, and Lipton SA. Dominant-interfering forms of MEF2 generated by caspase cleavage contribute to NMDA-induced neuronal apoptosis. *Proc Natl Acad Sci U S A* 99: 3974–3979, 2002.
65. Pan Z-H, Bähring R, Grantyn R, and Lipton SA. Differential modulation by sulfhydryl redox agents and glutathione of GABA- and glycine-evoked currents in rat retinal ganglion cells. *J Neurosci Res* 15: 1384–1391, 1995.
66. Pellegrini JW and Lipton SA. Delayed administration of memantine prevents NMDA receptor-mediated neurotoxicity. *Ann Neurol* 33: 403–407, 1993.
67. Qu J, Nakamura T, Cao G, Holland EA, McKercher SR, and Lipton SA. S-Nitrosylation activates Cdk5 and contributes to synaptic spine loss induced by β -amyloid peptide. *Proc Natl Acad Sci U S A* 108: 14330–14335, 2011.
68. Satoh T, Akhtar MW, and Lipton SA. Combatting oxidative/nitrosative stress with electrophilic counterattack strategies. In: *Oxidative Stress and Redox Regulation*, edited by Jakob UH and Reichmann D. P Springer (In Press).
69. Satoh T, Kosaka K, Itoh K, Kobayashi A, Yamamoto M, Shimotojo Y, Kitajima C, Cui J, Kamins J, Okamoto SI, Izumi M, Shirasawa T, and Lipton SA. Carnosic acid, a catechol-type electrophilic compound, protects neurons both *in vitro* and *in vivo* through activation of the Keap1/Nrf2 pathway via S-alkylation of targeted cysteines on Keap1. *J Neurochem* 104: 1116–1131, 2008.
70. Satoh T and Lipton SA. Redox regulation of neuronal survival mediated by electrophilic compounds. *Trends Neurosci* 30: 37–45, 2007.
71. Satoh T, Okamura S-i, Cui J, Watanabe Y, Furuta K, Suzuki M, Tohyama K, and Lipton SA. Activation of the Keap1/Nrf2 pathway for neuroprotection by electrophilic phase II inducers. *Proc Natl Acad Sci U S A* 103: 768–773, 2006.
72. Satoh T, Razie T, Seki M, Tabuchi T, Kitagawa T, Yanagitai M, Senzaki M, Kosegawa C, Taira H, McKercher SR, Hoffman JK, Roth GP, and Lipton SA. Dual neuroprotective pathways of a pro-electrophilic compound via HSF-1-activated heat-shock proteins and Nrf2-activated phase 2 antioxidant response enzymes. *J Neurochem* 119: 569–578, 2011.
73. Stamler JS, Toone EJ, Lipton SA, and Sucher NJ. (S)NO signals: translocation, regulation and a consensus motif. *Neuron* 18: 691–696, 1997.
74. Stieg PE, Sathi S, Warach S, Le DA, and Lipton SA. Neuroprotection by the NMDA open-channel blocker memantine in a photothrombotic model of cerebral focal ischemia in neonatal rat. *Eur J Pharmacol* 375: 115–120, 1999.
75. Sucher NJ and Lipton SA. Redox modulatory site of the NMDA receptor-channel complex: regulation by oxidized glutathione. *J Neurosci Res* 30: 582–591, 1991.

76. Sucher NJ, Wong LA, and Lipton SA. Redox modulation of NMDA receptor-mediated Ca^{2+} flux in mammalian central neurons. *NeuroReport* 1: 29–32, 1990.
77. Sullivan JM, Traynelis SF, Chen HSV, Escobar W, Heinemann SF, and Lipton SA. Identification of two cysteine residues that are required for redox modulation of the NMDA subtype of glutamate receptor. *Neuron* 13: 929–936, 1994.
78. Takahashi H, Shin Y, Cho S-J, Zago WM, Nakamura T, Gu Z, Ma Y, Furukawa H, Liddington RL, Zhang D, Tong G, Chen H-SV, and Lipton SA. Hypoxia enhances S-nitrosylation—mediated NMDA receptor inhibition via a thiol oxygen sensor motif. *Neuron* 53: 53–64, 2007.
79. Tenneti L, D'Emila DM, and Lipton SA. Suppression of neuronal apoptosis by S-nitrosylation of caspases. *Neurosci Lett* 236: 139–142, 1997.
80. Tenneti L, D'Emila, Troy CM, and Lipton SA. Role of caspases in N-methyl-D-aspartate-induced apoptosis in cerebrocortical neurons. *J Neurochem* 71: 946–959, 1998.
81. Tenneti L and Lipton S. Involvement of activated caspase-3-like proteases in N-methyl-D-aspartate-induced apoptosis in cerebrocortical neurons. *J Neurochem* 74: 134–142, 2000.
82. Uehara T, Nakamura T, Yao D, Shi Z-Q, Gu Z, Masliah E, Nomura Y, and Lipton SA. S-Nitrosylation of protein-disulphide isomerase links protein misfolding to neurodegeneration. *Nature* 441: 513–517, 2006.
83. Wang Y, Eu J, Washburn M, Gong T, Chen H-SV, James WL, Lipton SA, Stamler JS, Went GT, and Porter S. The pharmacology of aminoadamantane nitrates. *Curr Alzheimer Res* 3: 201–204, 2006.
84. Xia P, Chen H-SV, Zhang D, and Lipton SA. Memantine preferentially blocks extrasynaptic over synaptic NMDA receptor currents in hippocampal autapses. *J Neurosci* 30: 11246–11250, 2010.
85. Yao D, Gu Z, Nakamura T, Shi Z-Q, Ma Y, Gaston B, Palmer LA, Rockenstein EM, Zhang Z, Masliah E, Uehara T, and Lipton SA. Nitrosative stress linked to sporadic Parkinson's disease: S-Nitrosylation of parkin regulates it E3 ligase activity. *Proc Natl Acad Sci U S A* 101: 10810–10814, 2004.
86. Yeh M, Kaul M, Zheng J, Nottet HSLM, Thylin M, Gendelman HE, and Lipton SA. Cytokine-stimulated, but not HIV-infected, human monocyte-derived macrophages produce neurotoxic levels of L-cysteine. *J Immunol* 164: 4265–4270, 2000.
87. Zhao Y, Navia BA, Marra CM, Singer EJ, Chang L, Berger J, Ellis RJ, Kolson DL, Simpson D, Miller EN, Lipton SA, Evans SR, Schifitto G; the Adult AIDS Clinical Trial Group (ACTG) 301 Team. Memantine for AIDS dementia complex: open-label report of ACTG 301. *HIV Clin Trials* 11: 59–67, 2010.

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Abbreviations Used

Cdk5 = cyclin-dependent kinase 5
Drp1 = dynamin-related protein 1
ER = endoplasmic reticulum
Keap1 = Kelch-like ECH-associated protein 1
MMPs = matrix metalloproteinases
Nrf2 = nuclear factor erythroid 2-related factor 2
PDI = protein-disulfide isomerase
Prx2 = peroxiredoxin 2
SNO = S-nitrosothiol
XIAP = X-linked inhibitor of apoptosis