Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Physiology

# Cysteine oxidation and redox signaling in dopaminergic neurons physiology and in Parkinson's disease

Chiara Milanese[1], César Payán-Gómez[1,2] and Pier G Mastroberardino[1,3]

Parkinson's disease (PD) is a neurological disorder affecting dopaminergic neurons in the nigrostriatal pathways of the brain. PD is a multifactorial disease and its causes should be sought in detrimental interactions between genes and environment. Since early mechanistic studies, excessive oxidation – or oxidative stress – emerged as a recurring and fundamental pathogenic mechanism, and consequently received significant attention. More recent evidence obtained at single-cell resolution, however, indicates that dopaminergic neurons in the substantia nigra display increased oxidation levels also in normal, physiological conditions; differently than pathological oxidation, the importance of this phenomenon is underappreciated. The nigrostriatal dopaminergic system is involved in behavioral strategies that have been under strong evolutionary pressure. It is therefore improbable that physiological oxidation in dopamine neurons is accidental. Here, we review recent literature to argue that moderate oxidation improves redox signaling – which in dopamine neurons is intertwined with electrophysiological activity and is important to regulate dopamine release – and also has a protective role. We also reason that physiological oxidation provides an example of antagonistic pleiotropy therefore offering an advantage during reproductive stages of life while becoming detrimental during aging. Collectively, we believe that these observations provide a new perspective in the biology of dopaminergic neurons and in PD.

**Addresses**
[1] Department of Molecular Genetics, Erasmus MC, Rotterdam, The Netherlands
[2] Facultad de Ciencias Naturales y Matemáticas, Universidad del Rosario, Carrera 24, 63C-69 Bogotá, Colombia
[3] Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy

Corresponding author:
Mastroberardino, Pier G (p.g.mastroberardino@erasmusmc.nl)

**Current Opinion in Physiology** 2019, **09**:73–78

This review comes from a themed issue on **Redox regulation**

Edited by **Miriam Cortese-Krott** and **Sruti Shiva**

https://doi.org/10.1016/j.cophys.2019.04.025



Parkinson's disease (PD) is a neurodegenerative disorder primarily affecting dopaminergic neurons in the nigrostriatal pathways of the brain. Dopaminergic loss displays anatomical specificity and is more pronounced the lateral ventral tier of the *substantia nigra pars compacta* (SNpc); dopamine neurons in the ventral tegmental area (VTA) are relatively spared [1]. PD etiopathogenesis is complex and stems from detrimental synergies between genetic and environmental factors, ultimately perturbing crucial processes in the cell. PD is largely sporadic and monogenic forms represent only 5% of total cases [2]. Studies on monogenic PD, however, have been instrumental to unravel pathogenic mechanisms. At present, PD has been associated with 19 loci, which in turn have been unambiguously assigned to 11 genes (PD genetics has been reviewed in several excellent articles, e.g. Ref. [3]). The latter are involved in different biological processes, from protein quality control to endocytic trafficking, to redox homeostasis, therefore reinforcing the complexity of PD etiopathogenesis.

## Oxidation and PD

Despite the recognized intricacy of PD pathobiology, however, oxidative stress received continued attention since the seminal work of Langston *et al.* [4] describing parkinsonism in young subjects intoxicated with MPTP. Follow-up studies, in fact, demonstrated a direct inhibitory effect of MPTP on mitochondrial respiratory complex I with consequent increase in reactive oxygen species (ROS) production [5]. After its detection in patients' specimens, oxidative stress rapidly became regarded as a highly plausible PD mechanism [6,7]. Importantly, alterations in redox homeostasis have been detected in animal models harboring mutations in PD-associated genes [8]. Additionally, PD modeling largely relies on induction of oxidative stress and virtually all the accepted toxicological models are based on chemicals that ultimately function as pro-oxidants [9]. Oxidative stress, therefore, remains a recurrent factor of both genetic and idiopathic PD, and a point of convergence in the pathogenic cascade. More recently, the concept of oxidative stress in PD evolved in light of the crucial role of oxidation in normal biological function, where physiological alterations of the intracellular oxido-reductive (redox) state modify sensitive residues in proteins to modulate their activity [10]. The process largely operates via reversible oxidation of thiols in cysteine residues and is referred to as thiol redox signaling.

The topic of oxidative stress and redox signaling in neurodegeneration and in PD has been comprehensively reviewed in several and even very recent articles, and it is not our intention to revisit the information provided in

these excellent publications [11–13]. We would rather like to emphasize few underappreciated aspects concerning the redox metabolism of dopaminergic neurons in normal conditions and the potential physiological consequences.

## Oxidation and dopaminergic neurons

Dopaminergic neurons have distinctive redox properties. Here, a first supportive evidence comes from studies in toxicological models of PD, which clearly show that selective dopaminergic degeneration can be elicited not only by molecules specifically targeting dopaminergic neurons (e.g. MPTP), but also by chemicals acting systemically, for example rotenone and paraquat [14,15]. Another important evidence comes from studies that measure the intracellular redox state of dopaminergic neurons. Guzman *et al.* [16••] used a redox-sensitive green fluorescent protein (roGFP) [17] to demonstrate that in brain slices, in normal conditions (i.e. in the absence of pathology), dopamine neurons in the SNpc are more oxidized than those in the VTA. roGFP equilibrates – very slowly [18] – with the GSH/GSSG redox couple. Here, it should be emphasized that redox homeostasis relies also on additional couples, for instance thiols in thioredoxins [19,20], and that these systems are not at equilibrium, that is oxidation in one couple does not necessarily implies oxidation in the other [21]. Information inferred with roGFP is therefore necessarily limited to a specific aspect of the intracellular redox state. In a parallel approach, we took advantage of thiol-specific probes conjugated to fluorescent dyes to achieve differential labeling of oxidized and reduced cysteines to infer the global thiol/disulfide redox state in dopaminergic neurons at single cell level [22,23••]. Our experiments confirmed higher oxidation in dopaminergic neurons of the SNpc when compared to those of the VTA or to other neurochemical subtypes of neurons in the cortex [23••]. While the method we developed and used in these measures cannot discriminate between specific redox couples, it provides an overview of the general redox state of the cell. In combination with the results of Guzman *et al.*, our study provides converging evidence that the thiol/disulfide redox state is oxidized in dopaminergic neurons, in normal conditions, without ongoing pathology.

## Which are the causes underlying increased oxidation?

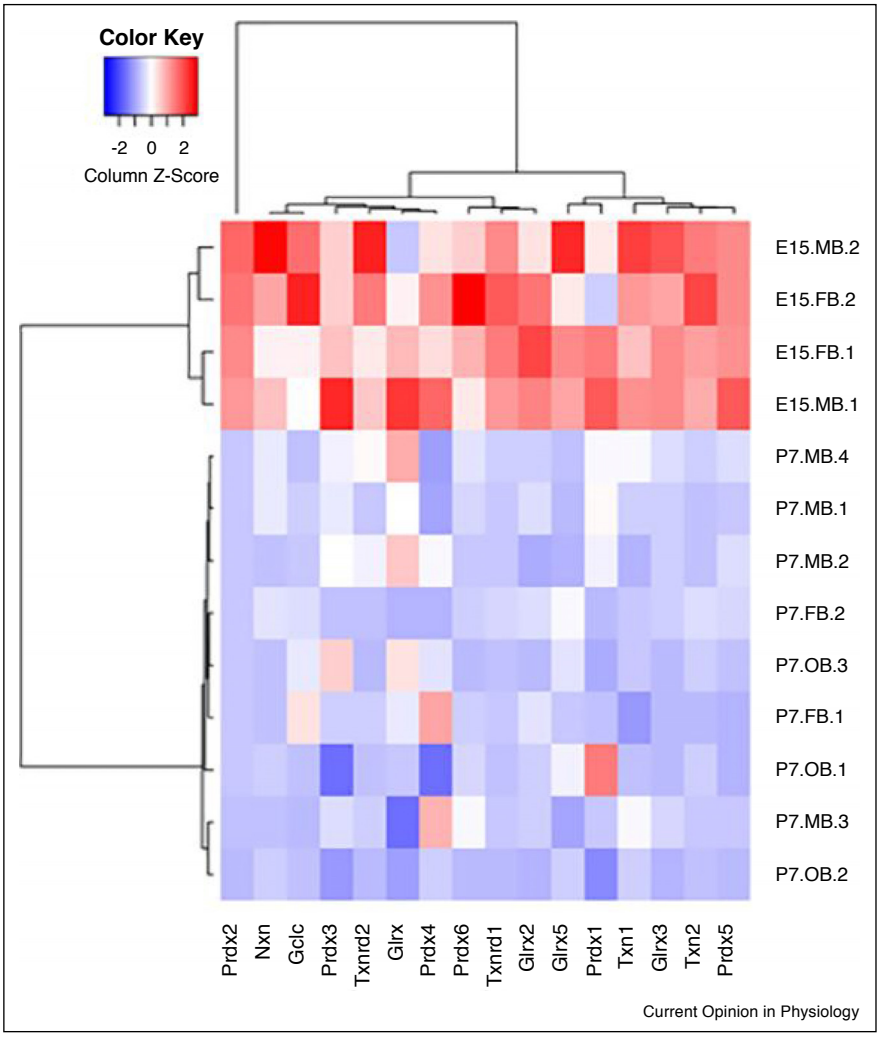
Increased oxidation in the thiol/disulfide redox couple in dopaminergic neurons is consistent with other elements. Some evidence indicates that expression of ROS scavenging enzymes catalase, Cu/Zn SOD dismutase, and glutathione peroxidase is lower in the SNpc than in the VTA [24,25]. To refine those observations, we took advantage of publicly available datasets from transcriptomic studies performed at single-cell level [26] to explore the expression levels of key genes in redox metabolism in embryos and seven days old mice. We found that expression of redox genes significantly decreases after birth; however, we could not detect major visible differences between dopaminergic neurons in the SNpc and those in the VTA (Figure1). These data do not exclude differences, which could be detectable at protein level, but certainly suggest that there are no ostensible transcriptional differences in SNpc dopaminergic neurons.

The SNpc also contains high iron levels [27], which can induce oxidation, especially in combination with $H_2O_2$ via Fenton chemistry [6]. Because $H_2O_2$ is abundantly produced by the enzyme monoamine oxidase during dopamine degradation, dopaminergic neurons in the SNpc appear to be particular inclined to oxidation. Finally, high production of ROS has been also attributed to the distinctive electrophysiological properties of SNpc dopaminergic neurons. Some studies indicate that pacemaking activity (described below in the text) depends on calcium influx through voltage-dependent Cav1.3 channels, and that calcium is buffered by mitochondria, which in turn produce superoxide during the process [18,28•]. For sake of completeness, it should also mentioned that other experiments attribute a less prominent role to calcium in dopamine neurons pacemaking activity especially in aging, which remains PD major risk factor [29,30]. Nonetheless, data generated independently, using different approaches, are consistent with the observation that the thiol/disulfide redox state in SNpc dopamine neurons is more oxidized than in other neuronal subtypes in normal conditions.

## Oxidation and evolution

Unlike oxidative stress in PD, the concept that SNpc dopamine neurons display increased oxidation also in normal conditions has not received commensurate attention. Yet, this is an important issue because it is highly conceivable that a basal oxidized redox state underlies the particular vulnerability of SNpc dopamine neurons to pro-oxidants, therefore predisposing to PD pathogenesis. Here, it should also be emphasized that available evidence indicates that SNpc dopamine neurons do not put in place mechanisms to mitigate oxidation in normal conditions, as inferred from level previous literature and from transcriptomic analysis at single-cell level [26] of major redox genes (Figure 1). It is very unlikely that this distinctive biochemical characteristic is accidental. The dopamine system is in fact essential to adapt behavioral strategies to environmental stimuli: given an external input, it has a role in selecting the most appropriate motor program and in learning new advantageous schemes. Simply put, the dopamine system is crucial to learn how to discriminate between positive and negative stimuli, and what actions should be put in place to take advantage of beneficial situations while

**Figure?1**




Two-way cluster analysis of single cell next generation RNA sequencing data (GSE108020) [26] from embryonic (E15) and post-natal (p7) mice illustrating expression levels of major genes involved in redox metabolism. While expression of redox genes is clearly higher in embryonic neurons, in post-natal specimens no obvious differences can be appreciated between different areas, including between the SN (MB.4) and the VTA (MB.1). ***Abbreviations for E15***: FB.1: forebrain neuroblast; FB.2: post-mitotic forebrain tyrosine hydroxylase-positive ($Th^+$) neurons; MB.1: midbrain neuroblast; MB.2: post-mitotic midbrain DA neuron. ***Abbreviations for P7***: FB.1: arcuate nucleus neuroendocrine $Th^+$ neurons; FB.2 mixture of arcuate nucleus $Th^+$ subtypes; MB.1: ventral tegmental area; MB.2: postnatal neuroblast; MB.3: periaqueductal gray area; MB.4: *substantia nigra*; OB.1: least mature $Th^+$ neurons; OB.2: progressively maturing $Th^+$ neurons; OB.3: most mature $Th^+$ neurons. ***Genes abbreviations***: *Prdx1-6*: peroxiredoxin 1–6; *Nxn*: nucleoredoxin; *Gclc*: γ-glutamylcysteine synthetase, glutamate cysteine ligase; *Txnrd1-2*: thioredoxin reductase 1–2; *Glrx1-5*: glutaredoxin 1–5; *Txn1-2*: thioredoxin1-2.

avoiding dangerous ones [31,32•]. It is therefore obvious that the dopamine system has been under strong evolutionary pressure. The question is therefore why dopamine neurons evolved with a physiologically oxidized intracellular environment despite this feature poses risks for neurodegenerative diseases. Obviously, it is highly plausible that this question has multiple answers. One possibility, however, is that increased oxidation is functional for intense redox signaling, which is required in dopamine neurons in the SNpc to fulfil their physiological properties.

## Physiology of DA neurons and redox signaling

Activity of dopaminergic neurons and subsequent regulation of dopamine release is a complex topic that has been reviewed in several excellent articles [31,32•,33]. For the purposes of this review, it is sufficient to provide a succinct overview of the process. Dopamine neurons display two dominant activity patterns (i.e. firing patterns), the tonic and the phasic modes. The tonic mode is characterized by spontaneous, regular activity that is associated with a steady level of dopamine, which is necessary to maintain normal function in the related

circuits [34,35]. In contrast, the phasic mode is characterized by sharp activity changes, that is bursts, that cause large changes in dopamine levels and may be initiated by different types of reward related stimuli [34–36]. The phasic mode is therefore highly relevant from the standpoint of the behavioral response. Among the various factors contributing to bursting control, at least by two types of ionic channels have a relevant role in the process: the ATP-sensitive potassium (K-ATP) channels and NMDA receptors, which can also act in concert to potentiate tonic firing [37,38••].

K-ATP channels are octameric complexes composed of four potassium inwardly rectifying channels (Kir6.X, typically Kir6.2 in neurons [39]) forming the pore, and four sulfonylurea receptor units (SUR1 or SUR2) that constitute the regulatory units [40]. Opening of K-ATP channels can be elicited by multiple factors associated with the metabolic status of the cell (reviewed in Ref. [41]) and causes membrane hyperpolarization, which in turn culminates in reduced electrical activity. This mechanism can serve as a neuroprotective strategy in conditions of metabolic distress such as hyperactivity during seizure [42,43] or excitotoxicity [44]. In SNc dopaminergic neurons, redox activation – for instance following $H_2O_2$ mediated opening of K-ATP – emerged as an important mechanism to regulate dopamine release [45••,46•]. The mechanism operates via modification of redox sensitive cysteine residues [47], is mediated by the regulatory subunit SUR1 [45••], and one study identified at least two-specific residues in the regulatory subunit SUR1 via site-directed mutagenesis [48]. Because expression of SUR1 has been associated with metabolic sensitivity and predisposition to dopaminergic degeneration, and in light of the distinctive redox properties of SNpc dopamine neurons, these findings are highly relevant for both dopamine neurons physiology and PD [49,50].

Also, NMDA receptors, which mediate calcium influx in the cell, can contribute to SNpc dopamine neurons' bursts in phasic mode. NMDA receptor activation alone, however, is not sufficient to switch neurons to the phasic mode and require other hyperpolarizing currents, for instance upon extrusion of sodium ions [51], or by K-ATP channel activation, which potentiate phasic burst firing [38••]. Also NMDA receptors are redox regulated and oxidation of sensitive cysteine residues inactivates the channel [52,53]. Thus, while both NMDA receptors and K-ATP channels sense the surrounding redox state, oxidation elicits opposite consequences. The physiological consequences of the different redox behavior of these channels will have to be addressed in future studies; however, the combination of redox mediated closure of NMDA receptor and K-ATP channel opening may prevent excitotoxicity while contrasting overexcitability, and may reflect a concerted strategy to protect against oxidative stress.

## Thiol/disulfide redox state and $H_2O_2$ signaling

Collectively, the discussed findings highlight the relevance of $H_2O_2$ signaling for the physiology of SNpc dopaminergic neurons and its importance in governing behavioral aspects that have been highly exposed to evolutionary pressure (also discussed in Ref. [28•]). The question is whether increased intracellular oxidation in the thiol/disulfide network could be beneficial for this process.

The mechanics governing $H_2O_2$ redox signaling are complex and some of their aspects of are only rudimentarily understood (reviewed in Ref. [54•]). It is for instance unclear how specificity is ensured in $H_2O_2$ signaling and in particular which chemical, biological, and structural criteria drive targeted modification of a certain cysteine residue. Another major issue stems from the very modest reactivity of protein thiols toward $H_2O_2$ ($k \sim 10^1$–$10^2\,M^{-1}\,s^{-1}$) [55]. How can cysteine modification occur in a time frame compatible with neuronal physiological needs? It could be argued that the reaction efficiency would be highly improved in proximity of $H_2O_2$ sources, where local concentrations are particularly high. At least in the case of K-ATP channels, however, some evidence indicates that $H_2O_2$ signaling originates from mitochondria rather than plasma membrane proteins such as NADPH oxidase [46•], and proximity between $H_2O_2$ and its target is therefore questionable. Further complication arises from the far higher rate constants ($k \sim 10^5$–$10^8\,M^{-1}\,s^{-1}$) of $H_2O_2$ scavenging enzymes – for instance peroxiredoxins (Prxs) – which are generally abundantly expressed. Very recent evidence suggests that Prxs might mediate $H_2O_2$ signaling to overcome low rates of reaction and lack of specificity [56••]; nonetheless, it cannot be excluded *a priori* that Prxs may quench, or even neutralize, $H_2O_2$ function as second messenger.

We hypothesize that constitutively higher oxidation in the thiol/disulfide couple could favor $H_2O_2$ at least in three ways. (1) Oxidation in a subpopulation of intracellular thiols would increase the relative concentration of $H_2O_2$, therefore favoring its action as a second messenger. This hypothesis implies that factors such as the *in vivo* redox potential of protein thiols will determine which residues will be oxidized in basal conditions, and also implies that proteins such as K-ATP channels will remain in a reduced state. Redox proteomic studies will be necessary to address this possibility. Additionally, experiments in which measures of redox signaling effectiveness will be paralleled by rigorous measures of intracellular redox state a single cell level will be necessary to conclusively assess the effect of dopaminergic neurons basal oxidation on $H_2O_2$ as second messenger. (2) Increased cysteine oxidation could also block the active site of Prxs, which are particularly sensitive to $H_2O_2$ mediated thiol oxidation, as also indicated by redox proteomics studies [57,58]. (3) Multiple lines of evidence indicate that

bioavailability of ROS is higher in SNpc dopaminergic neurons because of higher production and/or lower scavenging. In this chemical context, an increase in reversible cysteine oxidation may represent a protective strategy against irreversible and more dangerous forms of oxidation caused by high ROS levels. Accordingly, we have recently shown that moderate, reversible oxidation protects the dopaminergic system in multiple animal models of PD [59].

## Are these observations important for PD?

Thiol/disulfide oxidation in normal conditions in SNpc dopamine neurons could be an example of antagonistic pleiotropy, that is a trait that is favorable during reproductive stages of life – for instance to improve novelty-induced exploration – and is therefore under evolutionary pressure, but becomes detrimental during aging [60,61]. Increased oxidation may provide the substrate for genetic and environmental factors to trigger dopaminergic degeneration. Moreover, some evidence indicates that moderate oxidation might be protective in PD and thus preservation of tolerable level of reversible cysteine oxidation may constitute an experimental therapy worth exploring.

## Conflict of interest statement

Nothing declared.

## Acknowledgments

PGM was partially supported by a Target Validation grant from the Michael J. Fox Foundation; CM was supported by a fellowship from the Fondazione Veronesi.

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Fearnley JM, Lees AJ: **Ageing and Parkinson's disease: substantia nigra regional selectivity**. *Brain* 1991, **114**:2283-2301.

2. Klein C, Westenberger A: **Genetics of Parkinson's disease**. *Cold Spring Harb Perspect Med* 2012, **2** a008888.

3. Reed X, Bandres-Ciga S *et al.*: **The role of monogenic genes in idiopathic Parkinson's disease**. *Neurobiol Dis* 2019, **124**:230-239.

4. Langston JW, Ballard P *et al.*: **Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis**. *Science* 1983, **219**:979-980.

5. Ramsay RR, Dadgar J *et al.*: **Energy-driven uptake of N-methyl-4-phenylpyridine by brain mitochondria mediates the neurotoxicity of MPTP**. *Life Sci* 1986, **39**:581-588.

6. Fahn S, Cohen G: **The oxidant stress hypothesis in Parkinson's disease: evidence supporting it**. *Ann Neurol* 1992, **32**:804-812.

7. Jenner P: **Oxidative stress in Parkinson's disease**. *Ann Neurol* 2003, **53(Suppl. 3)**:S26-S36 discussion S36-S28.

8. Varcin M, Bentea E *et al.*: **Oxidative stress in genetic mouse models of Parkinson's disease**. *Oxid Med Cell Longev* 2012, **2012**:624925.

9. Martinez TN, Greenamyre JT: **Toxin models of mitochondrial dysfunction in Parkinson's disease**. *Antioxid Redox Signal* 2012, **16**:920-934.

10. Winterbourn CC, Hampton MB: **Thiol chemistry and specificity in redox signaling**. *Free Radic Biol Med* 2008, **45**:549-561.

11. Chinta S, Andersen J: **Redox imbalance in Parkinson's disease**. *Biochim Biophys Acta* 2008, **1780**:1362-1367.

12. Garcia-Garcia A, Zavala-Flores L *et al.*: **Thiol-redox signaling, dopaminergic cell death, and Parkinson's disease**. *Antioxid Redox Signal* 2012, **17**:1764-1784.

13. Sbodio JI, Snyder SH *et al.*: **Redox mechanisms in neurodegeneration: from disease outcomes to therapeutic opportunities**. *Antioxid Redox Signal* 2019, **30**:1450-1499.

14. Betarbet R, Sherer TB *et al.*: **Chronic systemic pesticide exposure reproduces features of Parkinson's disease**. *Nat Neurosci* 2000, **3**:1301-1306.

15. McCormack AL, Thiruchelvam M *et al.*: **Environmental risk factors and Parkinson's disease: selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat**. *Neurobiol Dis* 2002, **10**:119-127.

16. •• Guzman JN, Sanchez-Padilla J *et al.*: **Oxidant stress evoked by pacemaking in dopaminergic neurons is attenuated by DJ-1**. *Nature* 2010, **468**:696-700.
This study uses a redox sensitive fluorescent protein to determine the glutathione redox state in dopaminergic neurons at single cell level and shows that dopaminergic neurons in the substantia nigra are more oxidized than those in the ventral tegmental area.

17. Hanson GT, Aggeler R *et al.*: **Investigating mitochondrial redox potential with redox-sensitive green fluorescent protein indicators**. *J Biol Chem* 2004, **279**:13044-13053.

18. Gutscher M, Pauleau AL *et al.*: **Real-time imaging of the intracellular glutathione redox potential**. *Nat Methods* 2008, **5**:553-559.

19. Lillig CH, Holmgren A: **Thioredoxin and related molecules—from biology to health and disease**. *Antioxid Redox Signal* 2007, **9**:25-47.

20. Hanschmann EM, Godoy JR *et al.*: **Thioredoxins, glutaredoxins, and peroxiredoxins—molecular mechanisms and health significance: from cofactors to antioxidants to redox signaling**. *Antioxid Redox Signal* 2013, **19**:1539-1605.

21. Trotter EW, Grant CM: **Non-reciprocal regulation of the redox state of the glutathione-glutaredoxin and thioredoxin systems**. *EMBO Rep* 2003, **4**:184-188.

22. Mastroberardino PG, Orr AL *et al.*: **A FRET-based method to study protein thiol oxidation in histological preparations**. *Free Radic Biol Med* 2008, **45**:971-981.

23. •• Horowitz MP, Milanese C *et al.*: **Single-cell redox imaging demonstrates a distinctive response of dopaminergic neurons to oxidative insults**. *Antioxid Redox Signal* 2011, **15**:855-871.
This study uses fluorescent labeled maleimide derivatives to label reduced and oxidized cysteines and to measure the global thiol/disulfide redox state. The study shows at single cell level that dopaminergic neurons in the substantia nigra are more oxidized than those in the ventral tegmental area.

24. Trepanier G, Furling D *et al.*: **Immunocytochemical localization of seleno-glutathione peroxidase in the adult mouse brain**. *Neuroscience* 1996, **75**:231-243.

25. Hung HC, Lee EH: **MPTP produces differential oxidative stress and antioxidative responses in the nigrostriatal and mesolimbic dopaminergic pathways**. *Free Radic Biol Med* 1998, **24**:76-84.

26. Hook PW, McClymont SA *et al.*: **Single-cell RNA-Seq of mouse dopaminergic neurons informs candidate gene selection for sporadic Parkinson disease**. *Am J Hum Genet* 2018, **102**:427-446.

27. Griffiths PD, Dobson BR *et al.*: **Iron in the basal ganglia in Parkinson's disease. An in vitro study using extended X-ray absorption fine structure and cryo-electron microscopy**. *Brain* 1999, **122**:667-673.

28. • Surmeier DJ, Obeso JA *et al.*: **Selective neuronal vulnerability in Parkinson disease**. *Nat Rev Neurosci* 2017, **18**:101-113.
A comprehensive review providing an excellent overview on Parkinson's disease pathogenesis. The article also includes a clear description of cell-autonomous factors, specific for dopamine neurons that may be responsible for PD pathogenesis.

29. Putzier I, Kullmann PH *et al.*: **Cav1.3 channel voltage dependence, not Ca2+ selectivity, drives pacemaker activity and amplifies bursts in nigral dopamine neurons**. *J Neurosci* 2009, **29**:15414-15419.

30. Branch SY, Sharma R *et al.*: **Aging decreases L-type calcium channel currents and pacemaker firing fidelity in substantia nigra dopamine neurons**. *J Neurosci* 2014, **34**:9310-9318.

31. Schultz W: **Multiple dopamine functions at different time courses**. *Annu Rev Neurosci* 2007, **30**:259-288.

32. • Bromberg-Martin ES, Matsumoto M *et al.*: **Dopamine in motivational control: rewarding, aversive, and alerting**. *Neuron* 2010, **68**:815-834.
An excellent review discussing midbrain dopaminergic neurons physiology under a behavioral perspective.

33. Rice ME, Patel JC *et al.*: **Dopamine release in the basal ganglia**. *Neuroscience* 2011, **198**:112-137.

34. Grace AA, Bunney BS: **The control of firing pattern in nigral dopamine neurons: burst firing**. *J Neurosci* 1984, **4**:2877-2890.

35. Grace AA, Bunney BS: **The control of firing pattern in nigral dopamine neurons: single spike firing**. *J Neurosci* 1984, **4**:2866-2876.

36. Schultz W: **Predictive reward signal of dopamine neurons**. *J Neurophysiol* 1998, **80**:1-27.

37. Zweifel LS, Parker JG *et al.*: **Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-dependent behavior**. *Proc Natl Acad Sci U S A* 2009, **106**:7281-7288.

38. •• Schiemann J, Schlaudraff F *et al.*: **K-ATP channels in dopamine substantia nigra neurons control bursting and novelty-induced exploration**. *Nat Neurosci* 2012, **15**:1272-1280.
The study demonstrates that K-ATP channels gate burst firing the dopaminergic neurons of the substantia nigra and contribute to novelty-dependent behavior.

39. Karschin C, Ecke C *et al.*: **Overlapping distribution of K(ATP) channel-forming Kir6.2 subunit and the sulfonylurea receptor SUR1 in rodent brain**. *FEBS Lett* 1997, **401**:59-64.

40. Aguilar-Bryan L, Clement JP *et al.*: **Toward understanding the assembly and structure of KATP channels**. *Physiol Rev* 1998, **78**:227-245.

41. Dragicevic E, Schiemann J *et al.*: **Dopamine midbrain neurons in health and Parkinson's disease: emerging roles of voltage-gated calcium channels and ATP-sensitive potassium channels**. *Neuroscience* 2015, **284**:798-814.

42. Yamada K, Ji JJ *et al.*: **Protective role of ATP-sensitive potassium channels in hypoxia-induced generalized seizure**. *Science* 2001, **292**:1543-1546.

43. Yamada K, Inagaki N: **Neuroprotection by KATP channels**. *J Mol Cell Cardiol* 2005, **38**:945-949.

44. Soundarapandian MM, Zhong X *et al.*: **Role of K(ATP) channels in protection against neuronal excitatory insults**. *J Neurochem* 2007, **103**:1721-1729.

45. •• Avshalumov MV, Chen BT *et al.*: **Endogenous hydrogen peroxide regulates the excitability of midbrain dopamine neurons via ATP-sensitive potassium channels**. *J Neurosci* 2005, **25**:4222-4231.
The study demonstrated for the first time that K-ATP channels in dopamine neurons can be regulated by endogenous hydrogen peroxide.

46. • Avshalumov MV, Bao L *et al.*: **$H_2O_2$ signaling in the nigrostriatal dopamine pathway via ATP-sensitive potassium channels: issues and answers**. *Antioxid Redox Signal* 2007, **9**:219-231.
An excellent review on $H_2O_2$ signaling, specifically in dopaminergic neurons.

47. Coetzee WA, Nakamura TY *et al.*: **Effects of thiol-modifying agents on KATP channels in guinea pig ventricular cells**. *Am J Physiol* 1995, **269**:H1625-H1633.

48. Jiang B, Tang G *et al.*: **Molecular mechanism for H(2)S-induced activation of K(ATP) channels**. *Antioxid Redox Signal* 2010, **12**:1167-1178.

49. Liss B, Bruns R *et al.*: **Alternative sulfonylurea receptor expression defines metabolic sensitivity of K-ATP channels in dopaminergic midbrain neurons**. *EMBO J* 1999, **18**:833-846.

50. Liss B, Haeckel O *et al.*: **K-ATP channels promote the differential degeneration of dopaminergic midbrain neurons**. *Nat Neurosci* 2005, **8**:1742-1751.

51. Johnson SW, Seutin V *et al.*: **Burst firing in dopamine neurons induced by N-methyl-D-aspartate: role of electrogenic sodium pump**. *Science* 1992, **258**:665-667.

52. Aizenman E, Lipton SA *et al.*: **Selective modulation of NMDA responses by reduction and oxidation**. *Neuron* 1989, **2**:1257-1263.

53. Brimecombe JC, Potthoff WK *et al.*: **A critical role of the N-methyl-D-aspartate (NMDA) receptor subunit (NR) 2A in the expression of redox sensitivity of NR1/NR2A recombinant NMDA receptors**. *J Pharmacol Exp Ther* 1999, **291**:785-792.

54. • Stocker S, Van Laer K *et al.*: **The conundrum of hydrogen peroxide signaling and the emerging role of peroxiredoxins as redox relay hubs**. *Antioxid Redox Signal* 2018, **28**:558-573.
An interesting and comprehensive review on $H_2O_2$ mediated redox signaling presenting elements in favor and against direct or peroxiredoxin-mediated modification of cysteines by $H_2O_2$.

55. Marinho HS, Real C *et al.*: **Hydrogen peroxide sensing, signaling and regulation of transcription factors**. *Redox Biol* 2014, **2**:535-562.

56. •• Sobotta MC, Liou W *et al.*: **Peroxiredoxin-2 and STAT3 form a redox relay for $H_2O_2$ signaling**. *Nat Chem Biol* 2015, **11**:64-70.
The study demonstrates a redox signaling mechanism, in which $H_2O_2$ mediated protein modification is transduced by peroxiredoxin.

57. Go Y-M, Chandler JD *et al.*: **The cysteine proteome**. *Free Radic Biol Med* 2015, **84**:227-245.

58. van der Reest J, Lilla S *et al.*: **Proteome-wide analysis of cysteine oxidation reveals metabolic sensitivity to redox stress**. *Nat Commun* 2018, **9**:1581.

59. Milanese C, Tapias V *et al.*: **Mitochondrial complex I reversible S-nitrosation improves bioenergetics and is protective in Parkinson's disease**. *Antioxid Redox Signal* 2018, **28**:44-61.

60. Williams GC: **Pleiotropy, natural selection, and the evolution of senescence**. *Evolution* 1957, **11**:398-411.

61. Kirkwood TB: **Understanding the odd science of aging**. *Cell* 2005, **120**:437-447.