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REVIEW ARTICLE

Dopamine and Aging: Intersecting Facets

C. David Rollo

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Abstract Aging encompasses life itself so understanding requires frameworks that forge unity amidst complexity. The free radical theory of aging is one example. The original focus on damage was augmented recently by appreciation that reactive oxygen and nitrogen species are essential to normal signaling and cell function. This paradigm is currently undergoing an explosive expansion fueled by the discovery that regulatory organization is a merry-go-round of redox cycling seamlessly fused to endogenous clocks. This might best be described as an "Electroplasmic Cycle." This is certainly applicable to dopaminergic neurons with their exceptional metabolic, electrical and rhythmic properties. Here I review normal aging of dopamine systems to highlight them as a valuable model. I then examine the possible integration of free radical and ion channel theories of aging. Finally, I incorporate clocks and explore the multifaceted implications of electroplasmic cycles with special emphasis on dopamine.

Keywords Dopamine · Aging · Longevity · Redox · Free radicals · Ion channels · Clocks · Regulation · Electroplasmic cycle · Evolutionary theory

Abbreviations

AT_{r1}	Angiotensin receptor 1
CBP	CREB binding protein
Cry	Cryptochrome
DA	Dopamine
DAT	Dopamine transport and reuptake protein
DA_{r1}	Dopamine receptor 1
DA_{r2}	Dopamine receptor 2
ER	Endoplasmic reticulum
GH	Growth hormone
GHRH	Growth hormone releasing hormone
GSH	Reduced glutathione
GSH-S-TR	Glutathione S-transferase
GSSG	Oxidized glutathione
HO-1	Heme oxygenase 1
HPA	Hypothalamic–pituitary–adrenal axis
HVA	Homovanillic acid
IGF-1	Insulin-like growth factor 1
L-DOPA	L-Dihydroxyphenylalanine
LTP	Long-term potentiation
MAO	Monoamine oxidase
NE	Norepinephrine
NO	Nitric oxide
NOS	Nitric oxide synthase
NOX	NAD(P)H oxidases
NPAS2	Neuronal PAS domain protein 2
PD	Parkinson's disease
Per	Period
PKA	Protein kinase A
RONS	Reactive oxygen and nitrogen species
SAM	S-adenosylhomocysteine
SN	Substantia nigra
SOR	Superoxide
SOD	Superoxide dismutase
SCN	Suprachiasmatic nuclei
SUR	Sulfonylurea receptors
TN	Tyrosine hydroxylase
VMAT2	Vesicular monoamine transporter 2

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C. D. Rollo (✉)
LSB-226, Department of Biology, McMaster University,
1280 Main St. West, Hamilton, ON, Canada L8S 4K1
e-mail: rollocd@mcmaster.ca

Dopamine and Aging: Elephant's Teeth?

Other than for humans and domesticated animals, nature is populated almost exclusively by youth. Senescence likely reflects declining natural selection with age, or even deleterious consequences arising from maximizing competitive early fitness at the price of subsequent decline (i.e., live harder, die younger) [1]. The longevity of elephants ultimately resides with the number of replacements for teeth needed to chew wood [2]. We too reflect an evolution that did not emphasize indefinite maintenance of permanent teeth or even dopaminergic function beyond an age of likely reproductive contributions. Despite the possibility that some neurons may live >100 years and neurogenesis can replace losses, aging reflects declining cognitive and motor functions related to many neuronal subtypes. Of these "*…the dopaminergic system seems to represent a life-terminating machination…*" [3]. Dopamine (DA) systems are not only quintessential biomarkers of aging their functional decline may constitute a core mechanism of aging itself.

Brain regions involved in motor functions are highly sensitive to oxidative stress and this increases with age [4–6]. Declining motor function (bradykinesia) is a robust hallmark of aging that reflects qualitative and quantitative changes in DA functions in the substantia nigra (SN) and striatum. The striatonigral system is most susceptible to loss of function in aging and degeneration in Parkinson's disease (PD) [7]. Cognitive, motivational and emotional declines trace to the mesolimbic DA system, which is less sensitive to losses.

Special aspects of DA systems relevant to oxidative stress and aging include generation of free radicals by monoamine oxidases (MAO) that function in DA degradation, potential toxicity of DA metabolic products, relatively low levels of antioxidant defenses in brain (especially catalase), low expression of growth factors in regions of high DA vulnerability (e.g., SN), high levels of iron associated with the SN with possible dysregulation of heme, exceptional metabolic rates of DA neurons (particularly in the SN) and abundance of oxidizable unsaturated fatty acids and DA [8–12]. Furthermore, tyrosine hydroxylase (TH), the rate-limiting enzyme for DA synthesis, is inhibited by oxidation [13].

DA neuronal loss was identified as a key factor in cognitive and motor declines in most early studies based on postmortem analyses. Loss of DA neurons with age also occurs in *Drosophila* [14]. In rats a 55% loss of TH activity in SN by 24 months of age was associated with a 59% increase in protein carbonyls. H_2O_2 decreased TH activity in direct proportion to carbonylation [15]. DA metabolites (e.g., DOPAC, homovanillic acid (HVA)) decline with age in rat nigrostriatal, mesocortical and hippocampal regions [16].

Declining DOPA decarboxylase in nigrostriatal and mesolimbic compartments contributed to 37% loss of DA in old rats. In the mesolimbic system axonal degeneration was associated with aggregation of phosphorylated TH, amyloid precursor protein and α-synuclein. Axonal degeneration contributed to loss of TH, L-dihydroxyphenylalanine (L-DOPA) and a 51% loss of DA in the ventral striatum despite no obvious neuro-degeneration. TH mRNA declined in ventral midbrain but protein levels did not. Aging impacted the mesolimbic system more than the nigrostriatal system, opposite to PD [17].

Early estimates of DA neuronal loss in normal human aging were variable but often substantial [5, 18–22]. Between 50 to 90 years of age brain weight declines 2–3% per decade. Generally, estimates of pigmented (neuromelanin) neuronal loss amounted to ~10% per decade culminating in deficits of 30–80% at advanced ages. DA markers, including TH, dopamine transport protein (DAT) and various receptors averaged ~6–10% loss per decade culminating in 40–50% loss between ages 18 and 88 years [5, 23, 24].

Backman and Farbe [25] estimated neuronal loss in the SN at ~3% per decade. They suggested that DA synthesis reflected cell numbers and that synaptic number declined from early adulthood on. Elderly humans (~81 years) showed marked microglial reactivity in SN pars compacta, which likely reflected neuronal death and reaction to neuromelanin. Extracellular deposits of neuromelanin correlated with immunoreactive microglia. Microglial are activated by diverse stimuli including toxins, pathogens, aberrant proteins and cells expressing apoptotic markers. Activated microglia may induce neurodegeneration via free radicals generated by NAD(P)H oxidases [24, 26].

Declining neuronal density in the SN correlated with PD symptoms in undiagnosed elders [27]. In a 2008 study a 36.3% loss of TH neurons and a 28.3% loss of neuromelanin cells were confirmed in the human SN between ages of 20–88 years. Remaining neurons were hypertrophied suggesting compensatory adjustments [28]. An earlier study also suggested that increased volume of melanin-positive neurons (by approximately a third) likely reflected compensation for neuronal losses [29].

Between 44 and 91 years of age a significant loss of TH neurons in the SNpc was detected in normal aging, but no loss was detected if centenarians were included [30]. TH levels in human synaptosomes from the caudate showed little change with age, but the oldest subject was 63 [31]. Compensation at the level of nerve terminals was suggested. Some older studies and an increasing number of more recent work detect little evidence of neurodegeneration in various brain regions, leading some to conclude that aging does not involve neuronal losses [see 5, 32–38]. Many of these studies pertain to the cortex and

hippocampus. Notably, a study of *Rhesus* monkeys found no losses in neocortex or hippocampus but losses were detected in the pars compacta of the SN [39].

Problems estimating neurodegeneration include post-mortem degradation of biomarkers like TH and DOPA decarboxylase, staining and visualization methods, and variation in ages, species, gender, culture or animal strains examined. Statistical resolution is weakened by high inter-individual variation respective to sample sizes. Aging is notoriously variable and biomarker variation may increase with age. Variable expression of biomarkers for DA neurons, including interplay of compensatory mechanisms and age-related inhibition further contribute. DA synthesis declines with age. TH also declines and may even be lost entirely. Alternatively, neuromelanin accumulates as an end product of DA degradation. DAT expression declines more than TH (which could help maintain DA levels).

Squirrel monkeys showed no overall changes in the number of cells expressing TH and neuromelanin in aged SN despite DA declines of 24%. In fact, cells expressing TH alone declined with age whereas those expressing neuromelanin alone increased [36]. Others similarly found that pigmented neurons in the SN of old monkeys were ~8-fold greater and unpigmented neurons were less than half those seen in young animals even though total neuronal complement showed no change [40]. Aged cells were more susceptible to the neurotoxin (MPTP), with TH-only cells being least vulnerable and neuromelanin-only cells being most [36]. Thus, neuromelanin is a biomarker of age-related defects, vulnerability to apoptosis, and reduced TH expression.

TH was absent in 8–11% of melanin staining cells in normally aged people (78–85 years old) and 4–9% in middle aged individuals (37–43 years old) [29]. The same phenomenon was reported in PD. Melanized neurons were most susceptible to loss in PD, and those surviving showed reduced TH mRNA. This likely reflected oxidative stress [41]. Remarkably ~8% of pigmented cells in the SN did not express TH. Decreases in TH mRNA were also associated with loss of protein [42]. Loss of TH in a subset of aging cells could contribute to DA loss and overestimates of neuronal losses.

Despite uncertainty regarding neurodegeneration in normal aging from cell-counts, apoptotic biomarkers were not only expressed in ~2% of melanized neurons in normal human SN, fragments of melanized neurons were found in glia. The mitochondria of melanized neurons reflected oxidative stress [43]. Others found apoptosis in SN was detectable in both normal aging (0.2–1.8%) and PD (0.4–10.1%) [44]. Even such low apoptotic rates would accrue significant neuronal losses over time. A possible contribution of neurogenesis to neuronal complement was not considered in early studies.

Regardless of any contribution of neurodegeneration, functional declines in DA systems with age are extensively documented. Biomarkers of nigrostriatal dysfunction and motor impairment include increased MAO activity, decreased TH, declining DA synthesis, and insensitivity of adenylate cyclase to DA (receptor resistance). Alterations extend to DA catabolism, synaptic DA uptake, receptor turnover and numbers of DA receptors [45]. Early estimates suggested that DA levels decline by 50–60% in advanced normal aging [18, 46] whereas loss of DA neurons in PD amounts to 80–90% in the SN, 40–50% in the ventral tegmental area (VTA) and 2–3% in the central gray [47].

In review, Reeves et al. [5] estimated DA loss at 10–13% per decade, although some studies found no declines. Some suggested that DA deficiency sufficient to elicit PD symptoms would be expected in normal aging by 110–115 years [3, 46]. In a 2003 study DA levels decline threefold from a peak in caudate at 9 years old of 7.8 ng/mg wet mass to 2.3 ng/mg at 87 years of age. Putamen was similar [48]. This was closely paralleled by HVA levels. A 13% decline in TH was detected in caudate. DAT and vesicular monoamine transporter 2 (VMAT2) levels paralleled changes in TH. Loss of DA but relative preservation of associated proteins highlighted DA loss as a primary defect [48].

Old Rhesus monkeys (23–28 years old) showed 44% less HVA and 79% less DOPAC in the SN compared to 8–9 years old monkeys. D-amphetamine stimulation of DA was diminished by 30% even by middle-age (14–17 years) and by 67% in old animals [49]. Motor function showed nearly fivefold decline with age. Conservative estimates suggested that DA declined by 20% and 20% of TH-positive neurons were lost in the aged putamen. Only HVA proved significantly different in SN. Old animals averaged 26% fewer TH-positive neurons in the SN but individual variation was high [49]. TH mRNA declined by perhaps 80% from 20 to 30 years of age to 79 years [50].

Early estimates of DOPA decarboxylase suggested ~50% loss between the ages of 20 and 50 years. Kish et al. [51] re-examined this in light of PET studies suggesting little decrement in this biomarker of dopaminergic function in striatum [see 32]. DOPA decarboxylase measured in post-mortem striatum significantly declined between 30 and 87 years of age in caudate (27%), but a 16% loss in putamen was not statistically resolved. Imaging results of DOPA decarboxylase are more conservative than DAT or VMAT2 estimates of dopaminergic status probably due to compensatory mechanisms [51, 52].

Loss of DAT was estimated at ~10% per decade consistent with losses of nigrostriatal axons [5]. A precipitous decline (>95%) in SN DAT mRNA occurred beyond 57 years of age [50]. Human survivorship curves reflect

acceleration of age-related human mortality at age ~55. In some regions of the SN loss of DAT mRNA from ages of 20–70 years was as much as 75%. This traced to both cell losses and decreased expression. Losses in DAT exceeded declines in TH [53]. Age-related decline of DAT mRNA exceeded neuronal losses suggesting downregulation [23, 25] consistent with compensation to maintain synaptic DA.

PET analyses of DA function detected age-related declines in DA_{r1}, DA_{r2} and DAT, although measures of DA metabolism were less clear [54]. SPECT analysis of DAT detected age-related loss of 8% per decade culminating in 51% loss between 18 and 83 years of age [55]. A subsequent SPECT study estimated declines at 6.6% per decade between the ages of 18–88 years. This culminated in a 48% loss in caudate and 45% loss in putamen [56]. VMAT2 (highly associated with DA in striatum) was examined in a PET study of normally aging and PD subjects. An advantage of this marker is that it is not strongly affected by factors altering DA synthesis or turnover (i.e., compensation). Normal aging revealed a 0.5% per y decline in VMAT2 binding sites in 75 subjects 20–79 years old. This culminated in ~30% decline in oldest ages. Trends were similar among striatal regions [52]. Results were also consistent with estimates of 70–80% DA loss in PD. Overall, PET/SPECT imaging for DAT and VMAT2 are highly consistent with estimates of dopaminergic system declines, including those from postmortem examinations.

DA receptor 2 (DA_{r2}) expression declines with age. Binding by DA_{r1} and DA_{r2} decline from early adulthood by 4–10% per decade. Generally, markers of DAT, DA_{r1} and DA_{r2} are highly correlated within and between caudate (cognition) and putamen (motor function) despite regional differences in function. DA_{r1} and DA_{r2} declined with age in many brain regions outside the striatum. These include frontal cortex (DA_{r1} and DA_{r2}), and for DA_{r2}, temporal and occipital cortices, hippocampus and thalamus [23, 25]. Earlier review placed DA_{r1} decline at ~7% per decade and DA_{r2} in the caudate at ~5% per decade [5]. The sensitivity of DA receptors (via adenylate cyclase) also declines with age (DA resistance). Exercise, dietary restriction, estrogen and prolactin increase DA_{r2} availability. Overall evidence suggests that measures improving DA transmission ameliorate age-related motor and cognitive declines [5].

Dopamine and Aging: Non-Motor Impacts

Besides mediating declining motor functions a plethora of age-related cognitive and emotional defects reflect dopaminergic dysregulation. These include visuospatial skills, interval timing, verbal skills, episodic memory (word, figure, facial recognition), attention, motivation, reasoning, executive functioning (visual working memory, verbal fluency) and even general intelligence [23, 25, 57]. Age-related declines in pre- and post-synaptic DA markers amount to 4–10% per y beyond age 20 [57]. DA_{r1} is particularly implicated in memory loss. A DA_{r1} agonist improved working memory in aged rhesus monkeys. Improvement was retained for a full year after treatment was discontinued suggesting that increasing DA_{r1} sensitivity may benefit normal aging and PD [58]. Regional DA_{r2} activity correlated with frontal cortex, cingulate and caudate striatum glucose metabolism.

Declining DA_{r2} is associated with motor performance and frontal cortex cognitive impairment. Both DA_{r2} and glucose metabolism declined with age, but the relationship remained significant after controlling for age [54, 59]. Age-related deficits in episodic memory, executive functions and intelligence test scores were associated with declines in DAT binding in the caudate and putamen [57]. Dopaminergic function contributes to cognitive performance independently of age [54, 57, 59]. Given that aging DA systems substantially impact cognition this should be considered in addition to contributions of more subtle age-related changes in other brain regions (e.g., hippocampus).

If age-related changes in DA functions are responsible for defects these may be more amenable to treatment than neuronal losses. This also has implications for therapies involving neurogenesis since precursors may encounter unfavorable microenvironments or a niche littered with surviving defective cells. The most likely alterations that could impact DA synthesis and signaling are probably oxidative stress and ionic imbalance. From an electroplasmic perspective this could entail mitochondria, NAD(P)H oxidases (NOX), microglial activation, distorted ion channel functions, nitric oxide synthase (NOS), dysregulated signaling networks (e.g., redox pathways, kinase-phosphatase systems, loss of clock functioning), proteosome inhibition, protein accumulation and aggregation, and antioxidant enzyme inhibition. DA systems sustain >80% loss before manifesting PD symptoms so age-related motor and cognitive declines presumably reflect substantial alterations. Resilience may reflect compensation or adaptive redundancy evolved to maintain peak functions at ecologically relevant ages.

Advances in identifying genes and mechanisms related to evolution of the human brain have rapidly accelerated [60]. DA relates to cognitive performance independently of age [57] suggesting the intriguing possibility that DA systems have been upregulated to obtain advantages critical to human evolution. Indeed several genes identified as contributing to human brain evolution include MAO-A and DA_{r4} associated with DA metabolism, emotionality and behavior [61]. Gene arrays show that human brains have higher rates of neuronal activity and gene transcription than chimpanzees or *Rhesus* macaques [62]. In particular,

increased cognitive power, precarious bipedal locomotion—including simultaneous tool use, and especially, sophisticated martial arts and weapons skills, all rely on dopaminergic underpinnings. DA antagonists impair cognitive and motor performance in monkeys [23]. Significantly, DA levels were 5–10 times higher in *Rhesus* monkeys than rats and HVA was more than 10-fold higher [49].

The exceptional metabolic rate of the SN correlates well with its enhanced susceptibility to functional losses in normal aging and PD [10]. Further, there is extensive reciprocal interconnection of the striatum and neocortex such that age-related cognitive changes mainly trace to striatal-frontal circuitry [23, 25]. It is noteworthy that extensive neuromelanin deposition in the substantia nigra is particularly accentuated in humans, and is not pronounced in other DA systems of the human brain. Thus, sensitivity of SN DA neurons to aging and pathology may represent a quintessential example of the antagonistic pleiotropy theory of aging—elevated youthful fitness with an aging price tag [63]. Of note, DA biomarkers in brain increase during childhood to age 9 [48]. This is consistent with theory that slow growth during extended childhood protects the brain from growth-related oxidative stress, and deferral of rapid growth to teenagers (another human-specific feature) does not involve brain [60]. Finally, declines in key DA markers begin in early adulthood [48] consistent with theory that aging begins at sexual maturity.

Dopamine and Longevity

Most models of extended longevity express general stress resistance [64, 65] and catecholamines mediate waking stress hormone axis functions. Tyrosine and DA levels rise with stress in *Drosophila* and are associated with longevity [66]. Treatment of PD remains largely limited to L-DOPA and various dopaminergic agents, but effectiveness declines with disease progression and side effects increase [67]. L-DOPA may accelerate progression via upregulating DA metabolism in a declining cell complement. In normal animals, however, up-regulation of DA or norepinephrine (NE) (peaks correlate with hypothalamic–pituitary–adrenal axis [HPA] activity) can improve longevity, restore growth hormone (GH) pulse amplitude and re-initiate estrous of old rodents [e.g., 68, 69].

The impact on GH pulse amplitude links age-related DA and GH axis declines. Indeed, L-DOPA stimulates release of growth hormone releasing hormone (GHRH) in humans [70]. Deprenyl increased DA ($\sim$3-fold) and TH in old rats, and restored plasma insulin-like growth factor 1 (IGF-1) to youthful levels [71]. Alternatively, in the tuberoinfundibular DA system, elevating IGF-1 protected DA neurons from age-related dysfunction and prevented associated hyperprolactemia in 28 months old rats [72]. Neuronal loss in aging untreated animals was modest but accelerated in old age. TH increased in treated senescent animals compared to untreated controls reflecting both increased neuronal numbers and functional integrity [72]. Such results suggest a linkage of DA and GH axis declines in aging and the possibility of a vicious feedback mechanism. Thus, DA loss could contribute to declines in GH pulsatility, and loss of GH axis function could exacerbate faster age-related loss in DA system function.

Feeding L-DOPA to mice (4–5 weeks old) prolonged their youthful phenotype, improved impregnation and reproduction at 12 months of age, and nearly doubled survivorship at 18 months [68]. L-DOPA may be a dietary restriction mimetic since it did not alter feeding despite reduced growth rates and respectable increases in maximal and mean longevities [69]. Disruption of DA_{r2} also reduced IGF-1 and GH secretion and creates a dwarf mouse [73]. The TH chromosomal region was linked to centenarians and suggested linkages between TH, insulin and IGF-2 in promoting longevity [74]. A crucial role of the GH axis and signaling by insulin and IGF-1 are well established [reviews 64, 65].

Deprenyl, strongly induces endogenous antioxidants, inhibits RONS generation by monoamine oxidase B, elevates DA levels and extends longevity [71, 75]. A strong case for DA as critically modulating aging and lifespan was suggested by improved cognitive aging and lifespan in rats associated with protection of nigrostriatal DA neurons by L-deprenyl [3]. Full sexual activity was restored in rats at ages that were otherwise nonfunctional. Interestingly, sexual activity, learning ability and longevity were strongly and positively correlated. Maintenance of basic physical performance in aging generally correlates to longevity in animals and humans [76] and treatment of aged rats with a DA receptor agonist (haloperidol) ameliorated age-related declines [77]. L-Deprenyl is ideal because it is stimulating, protective and selective for nigrostriatal DA [3].

DA is a prime candidate as an aging mechanism in insects [78]. Genetic variation at the DOPA decarboxylase locus (which directs the final step in DA and SRT synthesis) correlates with longevity of *Drosophila* [79]. *Drosophila* divergently selected for virgin longevity expressed increased DA and melanin pigmentation in short-lived lines. Melanin is produced by polymerization of the quinone products of DA and L-DOPA. This was associated with elevated locomotion and respiration rates consistent with the rate of living theory. Flies selected for extended longevity, however, showed no consistent changes in DA or melanin. Although they also expressed greater locomotion this was not associated with increased respiration rate [78]. DA levels showed strong gender differences with males expressing more DA than females.

Pigment dispersing factor mediates output from the *Drosophila* clock that regulates activity-rest cycles. Mutations in *Pigment-dispersing factor*, *Clock* and *Cycle* reduce periods of rest. Mutated *Cycle* caused particularly drastic reductions in rest and can induce mortality in association with sleep deprivation. Reduced rest likely involves DA since DA critically regulates arousal in *Drosophila*. Males may accumulate greater sleep debts that reduce longevity [80]. This resembles male marsupial mice that die following intense activity during their breeding period [81]. *Cycle* regulates both rest and male life span in the fly [80]. Mouse *Clock* mutants also show reduced sleep [82].

Aging female rats express declining hypothalamic catecholamines (DA, NE) and reduced secretion of DA from the median eminence. This was associated with cessation of estrous cycles [83]. L-DOPA restored estrous cycling and luteinizing hormone responses to estrogen in aged non-cycling rats [84]. Clonidine, an α-adrenergic agonist, also restored luteinizing hormone pulsatility in acyclic rats, highlighting a role of NE in reproduction as well [85]. DA and NE were lower in hypothalamus of 21 months old male rats compared to 3–4 months olds. DA was also lower in other brain regions, but not in the olfactory tubercle of old rats. Decreased release of gonadotropins and increased secretion of prolactin was associated with reduced metabolism of catecholamines and elevations in serotonin metabolism [86].

Growth of reproductive organs and reproductive activity of both genders of Japanese quail were enhanced by L-DOPA. Follicular size, egg number, egg size and body growth were all dramatically increased [87]. DA is well known to increase testosterone secretion. Serotonin [tryptophan feeding] may act antagonistically to DA with respect to reproductive regulation [86, 87]. Declining sexual function in male rats likely reflects central deterioration (including loss of gonadotropin releasing hormone neurons) rather than alterations in sex hormones. Sexually dysfunctional male rats (~19 months of age) showed ~50% loss of DA and HVA in the nucleus accumbens compared to 5 months old rats. NE was also significantly lower. Sexually active rats of the same age expressed little decline in DA or NE [88]. This highlights aging heterogeneity in overt behavior and DA function and reinforces the role of DA in male sexual function [3].

Age-related functional deterioration in the SN and to a lesser extent the mesolimbic system substantially impacts motor functions, motivation and arousal [see 9]. Alterations include progressive loss of spontaneous locomotion and motor activity, and decreasing levels of arousal and motivation relevant to athletics and exercise. An increasingly sedentary lifestyle is a primary cause of the metabolic syndrome (obesity, atherosclerosis, hypertension, insulin resistance, type II diabetes) and its extended risk profile. This includes oxidative stress involving NOX, dysregulated mitochondria, heart failure, stroke, dementia, cancer, inflammation and autoimmunity (i.e., accelerated aging). Hypertension, a biomarker of aging, can trace to oxidative dysregulation of DA receptors in proximal renal tubules associated with elevated NOX activity [89–91]. Increasing evidence suggests that cognitive declines may be offset by stimulation and challenge [92]. Declining DA function may literally dim the zest for life. Declining motivation to engage in active living likely effects a self-fulfilling prophesy (use it or lose it). Interventions that restore or maintain youthful levels of arousal, motivation cognition and motor function hold great promise [93].

Ion Channels in Aging and Neuropathology

"Gatekeepers of Life and Death" [94]

The foundations for a general "*Ion Channel Theory*" of aging have unfolded across decades of intense research into brain function, aging and pathology. A "Calcium dysregulation hypothesis" dates back to the early 1980s [95–97]. Ca^{2+} influx and release from intracellular stores contributes to neurotransmission and signaling, but mediates apoptosis at high levels [96, 98]. Besides alterations in aging, Ca^{2+} dysregulation precedes symptoms and links to major features of Alzheimer's, Huntington's and PD diseases [e.g., 96, 97]. Ca^{2+} is a kingpin in excitotoxicity implicated in various neurodegenerative conditions, including PD [96, 97, 99–101].

Ion channels are regulated by or regulate cellular redox and associated reactive oxygen and nitrogen species (RONS). In particular, many channels are modulated by reversible modification of redox-sensitive cysteine or methionine residues, often in conjunction with GSH/GSSG redox status [101–103]. Thus, free radical theories of damage and regulation seamlessly enfold an ion channel paradigm. It is not surprising that appreciation of ion channels centers in neuroscience since the exaggerated electrical activity of neurons requires dedication of most cellular ATP to channels [8].

Generally, RONS depolarize cell membranes via mechanisms involving Na^+, K^+ and Ca^{2+} [e.g., 102, 104]. Redox regulation of ion channels involves numerous mechanisms including transcription, trafficking, degradation and post-translational modifications (e.g., glutathionation, nitrosylation, and oxidation of key amino acid residues) [101, 102, 105, 106]. RONS modulation of MAPK pathways (upregulation of kinases, downregulation of phosphatases) can also ultimately impact channels, and channels themselves may be phosphorylated.

RONS promote Ca^{2+} entry and disrupt Ca^{2+} homeostasis in many cell types. For RONS-induced depolarization of endothelial cells, Ca^{2+} channel activation depends on levels of GSSG. GSH reversed GSSG-induced Ca^{2+} channel activation. Elevation of GSSG in oxidatively stressed cells likely reflects depletion of NAD(P)H reducing equivalents [104]. H_2O_2 generated by AMPA receptors in striatum suppressed axonal DA release, acting via ATP-sensitive K^+ channels (K_{ATP}) [107]. Glutamate can inhibit striatal DA release via receptor generation of H_2O_2 and opening of sulfonylurea-sensitive K_{ATP} channels, an impact opposed by $GABA_{rA}$ activation. Interestingly, there are no glutamate or GABA receptors on these DA fibers suggesting that H_2O_2 acts via diffusion from other neurons [107]. This is similar to the proposed role of H_2O_2 as an inter-cellular bystander signal in radiation biology [108]. H_2O_2 activates K_{ATP} channels by decreasing sensitivity to ATP. Impacts also depended on levels of ADP [109]. Thus, K_{ATP} channel activity likely depends on the ATP/ADP ratio as modulated by H_2O_2.

Ion Channels and General Brain Aging

Changes in ion channel activity are associated with age-related cognitive declines and RONS impacts on channels critically contribute to cell dysregulation or death [101, 102]. Aging rates have largely been approached from a vantage of oxidative stress, particularly that associated with mitochondria [110, 111]. Lutz et al. [112] proposed that exceptional longevity might be obtained by mechanisms protecting against anoxia-reoxygenation (ischemia-reperfusion) as in turtles. They highlighted regulation of ion channels under hypoxia. K^+ channels, particularly voltage-gated (Kv) and K_{ATP} channels are impacted by hypoxia. Kv channels are implicated in O_2-sensing [113].

This might generalize to other conditions of respiratory inhibition or energy depletion, virtually any condition decreasing mitochondrial ATP production or perhaps exhaustion by critical functions like DNA repair. Increasing longevity is strongly correlated with increasing body size and associated declines in mass-specific metabolic rate interspecifically. Across species, however, cells in culture show relatively similar respiration rate in air, despite declining rates with increasing body size in vivo [114]. Raising ambient O_2 increased respiration of cells in culture, suggesting that O_2 supply limits respiration in vivo. Thus, constraint on cellular respiration with increasing body size is reminiscent of hypoxia.

The situation is complicated since numerous features contribute to O_2 supply and demand (e.g., circulation rate, haemoglobin, mitochondrial number). A reliable way to increase diving duration in homeotherms is to simply increase body size, a circumstance that might explain the exceptional body sizes in cetaceans. Although such phenomena certainly reflect the association of increasing size with decreasing metabolic rate, they conversely highlight that there must be adaptations to lower oxygen environments that may hold the promise of extended longevity. Energy shortfalls particularly impact brain where maintenance of channels is most expensive, and dysregulation is most deleterious. The SN may be the quintessential example of a canary in a brain mind.

Alterations of Ca^{2+} and associated electrophysiological functions of neurons are reliable biomarkers of aging. Aging entails elevated Ca^{2+} influx and release from endoplasmic reticulum (ER), declining efflux, declining neuronal excitability, higher thresholds for long-term potentiation (LTP) and faster decay, enhanced long-term depression, increasing slow afterhyperpolarization, higher resting hyperpolarization, and rising Ca^{2+} with repetitive synaptic stimulation [e.g., 38, 96, 97, 115]. L-type Ca^{2+} channels increase in density [116], calcineurin shows increased phosphorylation and the Ca^{2+}-binding protein, calbindin-D_{28K}, declines. SN DA neurons containing calbindin-D_{28K} were more resistant to loss in PD, suggesting a protective role [117].

An L-type channel blocker or calcineurin inhibitor blocked the effects of both high (inhibitory) and low (stimulatory) H_2O_2 impacts on LTP [118]. Influx of Ca^{2+} through L-type channels was doubled in aged rats and was associated with age-related cognitive decline and AD. L-type channels mediate Ca^{2+} influx via NMDA receptors that can induce excitotoxicity. Increases in L-type $Ca_v1.2$ channel activity involved increased phosphorylation via cAMP-dependent protein kinase (PKA) [119]. K^+ channel activity also increases as cognitive ability declines and neuropathologies increase [116, 120–124].

Age-related Ca^{2+} dysregulation is associated with increasing RONS, toxicity associated with protein accumulation (especially α-synuclein, Aβ, and huntingtin) and metabolic dysregulation. This contributes to synaptic dysfunction, cognitive decline and neurodegeneration [96, 116, 125]. Age-related alterations in Ca^{2+}-regulating genes include increased expression of those involved in Ca^{2+}-dependent G-coupled signalling pathways [126]. Increases in L-type Ca^{2+} channels and other Ca^{2+} associated genes with age likely increase intracellular Ca^{2+} or turnover and inhibit neuronal activity [126].

L-type Ca^{2+} channels have redox-sensitive sulfhydryl groups. H_2O_2 enhances opening of voltage-gated Ca^{2+} channels, but high RONS levels are suppressive [101]. Enhanced Ca^{2+} entry likely explains increases in slow afterhyperpolarization which is mediated by Ca^{2+}-sensitive K^+ channels. Ca^{2+} channel blockers (e.g., the L-type Ca^{2+} channel antagonist, nimodipine) enhance associative and spatial learning in old animals [120, 127–129].

Synaptosomes of treated rats showed increases in the Ca^{2+}-binding proteins calbindin-D_{28K} and calreticulin in cerebral cortex [120]. Nimodipine improved motor performance on balance, hanging and climbing tests and age-related alterations in limb coordination were delayed [128]. Nimodipine also enhances cerebral blood flow and T- and L-type Ca^{2+} channel blockers are neuroprotective in cerebrovascular disorders [130, 131]. The relationship to cerebrovascular disorders and stroke relates to hypoxia and ischemia-reperfusion discussed above [112].

Intracellular Ca^{2+} release from ER regulates many cellular functions, and ER Ca^{2+} homeostasis is disrupted in many neuropathologies. Moreover, chemicals inducing parkinsonian syndromes elicit the unfolded protein stress response in ER [132]. Excitability of ER traces to Ca^{2+} pumps and release channels. Ca^{2+} release involves IP_3 and ryanodine receptors that are activated by Ca^{2+} influx (e.g., L-type channels) [123]. Ryanodine and IP_3 receptors bear numerous redox-sensitive cysteine residues. Low levels of H_2O_2 are stimulatory but high levels are inhibitory. GSH inhibits Ca^{2+} release [101].

The ER Ca^{2+} store contributes to neurotransmission (excitability and possibly neurotransmitter release and LTP). Ryanodine receptor type 3 activation via superoxide radical (SOR) and L-type Ca^{2+} channels was required for LTP in hippocampal slices [133]. Ca^{2+} release via ryanodine receptors is involved in the functioning of the SCN circadian clock and ryanodine receptor expression is itself circadian. Elevations in L-type Ca^{2+} channels could contribute to age-related ER dysregulation extending to cytosolic and mitochondrial buffers. Blockade of receptors with ryanodine reduced or eliminated age-related alterations in Ca^{2+} biomarkers [123]. RONS can also mediate ryanodine receptor release of Ca^{2+}. This can activate Ca^{2+} signalling pathways associated with synaptic plasticity. Alternatively, excess RONS may contribute to Ca^{2+} overload, neuronal dysfunction and apoptosis [134]. Overall low or acute Ca^{2+} signalling is essential for normal neuronal function but high and/or chronic Ca^{2+} elevations impair signalling, elevate oxidative stress and induce dysfunction.

Another approach to modulating Ca^{2+} is to manipulate K^+ channels. Ca^{2+}-dependent small voltage-gated K^+ channels (SK) modulate excitability of neurons including CA1 neurons in hippocampus. In aged rats, L-type Ca^{2+} channels impair LTP during high levels of synaptic activation in hippocampal slices (but facilitate LTD at low levels of synaptic activation). This involves afterhyperpolarization mediated by Ca^{2+}-dependent K^+ channels. The K^+ channel blocker apamin reduces after-hyperpolarization and promotes synaptic enhancement and learning [121, 135]. Substantial increases in transcription and SK potassium channel protein in hippocampus inhibited LTP and learning of mice. Blockade of these channels ameliorated age-related declines in LTP and learning [136] but mice over-expressing SK channels have severe learning disability. IP_3 receptor-evoked Ca^{2+} release causes SK channel-dependent hyperpolarization in prefrontal neurons. Blockade of the IP_3 receptor or SK channels (apamin) enhanced spatial learning and object recognition [137, 138]. Thus, elevation of SK channels traces to both L-type Ca^{2+} channel influx and Ca^{2+} release from ER.

Delayed rectifier-type K^+ channels are activated by depolarization and generally reduce excitation and facilitate repolarization. Age-related decreases in neuronal excitability can be offset by deletion of a Ca^{2+}-dependent K^+ channel modifier [129]. Deletion of the β1.1 K^+ channel subunit (Kvβ1.1) increased excitability of hippocampal CA1 pyramidal neurons and induced mild impairment of spatial and associative learning in young mice. In old mice, however, Kvβ1.1 knockout increased LTP and improved spatial learning [124]. Results varied with background strain highlighting the importance of genetic modifiers in brain aging [129]. A key regulator of longevity spanning nematodes to vertebrates is the forkhead transcription factor (FOXO) modulated by the PI3K signalling cascade. Targets of the *Caenorhabditis elegans* version, DAF16, included an aquaporin, voltage-gated L-type Ca^{2+} channel β-subunit and a voltage-gated K^+ channel [139]. Inactivation of the K^+ channel promoted a diapausing phenotype.

Dopamine and Channel Functions

Neurons in the SN contain NMDA receptors that receive glutamatergic inputs from the cerebral cortex and subthalamic nucleus. Activation of NMDA receptors causes Ca^{2+} influx and activation of NOS. NO can form highly toxic peroxinitrite and inhibition of NOS can be neuroprotective [99]. A key aspect of excitotoxicity and perhaps cell stress and apoptosis generally, is energy depletion. Altered metabolic and mitochondrial function in aging increases free radical processes, reduces energy production (ATP) and increases susceptibility to stressors. Thus, the defect in mitochondrial complex I in PD increases vulnerability to excitotoxicity [99]. HD is associated with DA receptor losses and impaired DA signalling. DA may also contribute to losses of crucial striatal neurons. Glutamate increased levels of intracellular Ca^{2+} and apoptosis of striatal medium spiny neurons (GABAergic) in huntingtin transgenic mice, but not in controls. Toxicity traced to mitochondrial Ca^{2+} overload and opening of the permeability transition pore. Blockade of the ER Ca^{2+} release receptor, IP_3R, mitochondrial Ca^{2+} uptake or the permeability transition pore were neuroprotective [100]. Mitochondria from a mouse model for Huntington's showed increased Ca^{2+}

sensitivity of the permeability transition pore, conferring high susceptibility to stressors mediating Ca^{2+} or free radical elevations [140].

Mouse Kv1.1 mediates amphetamine-induced hyperactivity and hypophagia [141] and Kv1.6 modulates DA release [142]. K^+ channels contribute to "channelopathies" including PD and manipulating K^+ channels may prove therapeutic [143]. Antibodies against voltage-gated K^+ channels were detected in encephalopathies with cerebral atrophy and cognitive dysfunction [144]. K_{ATP} channels are likely an element of stress responses as they are closely linked to voltage-gated channels and neurotransmission. Their expression is normally protective but chronic activation may be deleterious.

Electrical activity and associated aspects regulated by ion channels may crucially modulate DA neuronal survival [145]. TH expression and DA release are stimulated by electrical activity, and cells at high risk for degeneration show negligible TH expression. Thus, chronic hyperpolarization and diminished electrical activity may portend cell death. As with MPTP toxicity, elevated RONS may derive chronic K_{ATP} channel activity and hyperpolarization in these cells [145, 146]. K_{ATP} open in response to ATP depletion (hypoxia, excitotoxicity, mitochondrial dysfunction) causing K^+ efflux, hyperpolarization and loss of DA neuronal pacemaker function. Thus, K_{ATP} channels link cellular metabolic status (ATP/ADP ratio, O_2, glucose) to neuronal excitability [147, 148].

Various combinations of K_{ATP} subunits yield channels of differing sensitivity. K_{ATP} channels are composed of members of the inwardly rectifying Kir6 family and sulfonylurea receptors (SUR). DA neurons containing k6.2/SUR1 K_{ATP} channels expressed much greater rotenone-induced channel activation than neurons containing k6.2/SUR2B channels. Thus, reduced mitochondrial complex I activity in PD could differentially activate K_{ATP} channels. K_{ATP} channels are at the crux of a tradeoff between maintenance of membrane potential and contributing to excessive K^+ efflux under energy stress. Turtles and newborns have few K_{ATP} channels whereas neurodegeneration in the Weaver mouse is associated with highly sensitive K_{ATP} channels composed of k6.2/SUR1 subunits [147]. Vulnerability of DA neurons also correlates to regional metabolic rates.

Remarkably, H_2O_2 suppresses synaptic DA release and DA signalling even before cell losses accrue. Neurons in the substantia nigra were more susceptible than those in the nucleus accumbens or VTA [149–151]. Inhibition or depletion of H_2O_2 (via catalase) increased firing rates of DA neurons. Inhibition of GSH or elevation of H_2O_2 ultimately causes membrane hyperpolarization via opening of K_{ATP} channels [148]. In medium spiny neurons, rotenone-inhibition of mitochondrial complex I generates H_2O_2 which suppresses DA release via opening K_{ATP} channels [148, 151]. A role for H_2O_2 in regulating K^+ channels may reflect the value of H_2O_2 as a signal of mitochondrial functioning. A concentration threshold for H_2O_2 cellular impacts was suggested [148] consistent with a threshold for GSH depletion (and mitochondrial complex I dysfunction) noted by others [see 152]. Catalase activity is 20 times greater than that of GSH peroxidase in SN DA neurons which warrants closer scrutiny. With respect to regulation of K_{ATP} channels by H_2O_2, moderate doses may open these channels, but strong oxidants close them [148]. Significantly, highest K_{ATP} channel activity occurs in regions most susceptible to PD: SN and striatal areas regulating motor functions [148, 153].

A recent classic suggests that preponderance of L-type $Ca_v1.3Ca^{2+}$ channels makes DA pacemakers in the SN exceptionally vulnerable to stress and excitotoxicity [154]. These neurons are key victims of PD and L-type channels increase with age. Blockade of L-type Ca^{2+} channels with a drug used to treat hypertension and stroke (isradapine) caused neurons to assume a younger phenotype resistant to rotenone and MPTP [154]. This reinforces that blockade of L-type Ca^{2+} channels (or Ca^{2+}-sensitive SK channels) may offset age-related or pathological declines in DA systems.

Inactivation of voltage-dependent N- and P/Q-type Ca^{2+} channels is another possible target for RONS inactivation. DA_{r3} acts via the latter mechanism [155]. These authors suggest that DA_{r3} modulation of P/Q channels without affecting L-type Ca^{2+} channels would effectively prevent neuronal activation by low-level signalling/noise, providing cleared DA signal resolution. DA_{r2} can also selectively block N-type Ca^{2+} channels to reduce GABA release to rat striatal cholinergic interneurons [156].

DAT is a member of the Na^+/Cl^--dependent transporter family [157]. DA uptake by DAT is an "electrogenic" process that increases with hyperpolarization. Voltage dependence likely facilitates the ability of DA autoreceptors to terminate DA synaptic transmission [158]. DAT also eliminates extracellular DA in sleep. The lipid peroxidation product 4-hydroxynonenal is a cytotoxic aldehyde that has enormous redox implications because it binds cysteine redox switches [159]. DAT and Na^+/K^+ ATPase activity were strongly inhibited by 4-hydroxynonenal modification of their sulfhydryl groups [160].

The Na^+/K^+ ATPase pumps out Na^+ and pumps K^+ inwardly to modulate electrochemical gradients [161]. Inactivation by cysteine residue modification highlights that both DAT and Na^+/K^+ ATPase are regulated by redox state. DAT contains 13 cysteine residues [162]. Ectopic expression of DAT on non-DA cortical neurons facilitated uptake of DA and the toxin 6-hydroxydopamine. Toxicity was accompanied by a p38-induced K^+ efflux surge via voltage-gated (Kv) channels. Inhibition of DAT, p38 and

Kv channels were protective. This was also true of midbrain DA neurons strongly implying that DA neurodegeneration involves K^+ channels [163]. K^+ cellular efflux is a critical aspect mediating cell shrinkage, caspase activation and DNA fragmentation [164]. K^+ efflux via voltage-gated channels mediated glutamate toxicity in hippocampus [165].

DAT activity is suppressed by xanthine oxidase, H_2O_2 and quinones derived via DA metabolism [157, 159, 162]. DA metabolism generates ortho-quinone that can bind protein sulfhydryl groups on cysteine residues. Binding of DA metabolic products with protein cysteine residues represents a mechanism of DA toxicity potentially impacting global redox signalling and balance of cells (see below). Impacts include inhibition of enzyme functions (including DAT and ATPases), mitochondrial dysfunction, Ca^{2+} dysregulation, depletion of GSH and transcriptional alterations [159, 166]. Metabolism of DA also consumes considerable NAD(P)H reducing equivalents [166].

Besides inactivation, DAT declines with cell losses. DAT was not detectable in the putamen of PD victims and was reduced by 50–80% in the caudate [167]. Oxidative stress contributes to ionic dysregulation by disrupting ATPases (Na^+/K^+ ATPase, Ca^{2+} ATPase) and glutamate and glucose transporters. Ca^{2+} ATPase functions in Ca^{2+} export. Given the high cost of ion maintenance and reduced performance of aged mitochondria, prolonged depolarization can deplete ATP, compromise ATPases and promote Ca^{2+} overload [96]. Alternatively, Na^+/K^+ ATPase activity may contribute to neurodegeneration [161]. A mutation resulting in sustained hyperexcitation causes dystonia and parkinsonian symptomology [145].

Ion Channel Mutations and Altered DA in Mice

A plethora of mice expressing hyperactivity, circling or altered gate (and altered DA) as well as hyperactive "*Shaker*" *Drosophila*, have disrupted ion regulation tracing to mutations in Ca^{2+} pumps and K^+ channels [168–171]. K^+ channels impact DA, hyperactivity, sleep and longevity in *Drosophila* [171, 172]. Mice deficient in Kv3.1 exhibit hyperactivity and 40% less sleep, likely tracing to altered DA in the basal ganglia [173]. Ca^{2+} blockers also influence circling via impacting DA [131]. Many circling channel mutant mice with dysregulated DA and hyperactivity also have defects in vestibular regions and ear hairs rendering them deaf. Circling animals are often employed for testing dopaminergic drugs, including those relevant to PD.

Weaver mice develop DA neuronal losses and reductions in DA resembling PD: high losses in the SN and lower losses in the mesolimbic system. Cerebellar granule cells are also lost [10, 145]. *Weaver* mice have mutated G-coupled inwardly rectifying K^+ channel 2 (GIRK2) (= Kir3.2) which modifies K^+ efflux and membrane potential in response to neurotransmitters [161, 174]. Inappropriate Ca^{2+} entry could explain the 70-fold reduction in BDNF in the cerebellum of the *Stargazer* mutant mouse [175].

If Ca^{2+} can have such remarkable impacts on transcription, then it may also play a role in declining levels of TH and DA synthesis in normal aging. Lower levels of Ca^{2+} may be required to elicit DA release in SN compared to striatum [149]. Gene arrays of DA neuronal populations that are highly susceptible to loss (SN) versus neurons in the less susceptible ventral tegmental area (VTA) detected differential expression of many genes related to ion channel functions (e.g., calbindin, phospholipase A2, calcium ATPase, K^+ channels, Na^+ channels) [10]. K_{ATP} channel activity may counteract Ca^{2+} overload, excitotoxicity, and hypoxic stress. K_{ATP} channels expressed on DA neurons are likely linked to mitochondrial complex I inhibition and metabolic stress in PD. Inhibition of mitochondrial complex I in PD is generally found to be $\sim$40%. DA neurons expressing SUR-1 and Kir6.2 subunits are more sensitive to metabolic stress and these channels are activated in *Weaver* mice in response to mutated GIRK2 [147]. Mutated GIRK2 results in chronic Na^+ overload, depolarization and energy stress tracing to excessive ATP consumption by Na^+–K^+-ATPase. This then alters K_{ATP} channel activity. Inactivation of Kir6.2 ameliorates neurodegeneration in the SN implicating hyperpolarization as a factor in losses [146].

Neuronal Stress and Apoptosis: Beyond Calcium Dysregulation

Neuronal stress leading to dysregulation and death is reliably associated with a coordinated complex of mechanisms. These include Ca^{2+} influx, Ca^{2+} release from ER, intracellular Zn^{2+} release, K^+ efflux, mitochondrial dysfunction, oxidative stress, energy depletion (ATP) and oxidation of redox couples such as NAD(P)H/NADP and GSH/GSSH. Reliable associates include NOX activation mediating SOR and H_2O_2 generation, as well as Ca^{2+}-mediated generation of NO via NOS [176]. NO may interact with redox sensitive cysteine residues on diverse peptides [177]. Transition metals may further mediate formation of hydroxyl radical and peroxinitrite may arise from SOR interactions with NO. Hydroxyl radical and peroxinitrite are particularly toxic.

Oxidative stress and associated free radicals deplete GSH and when a critical loss of this buffer is reached ($\sim$50%) mitochondrial complex I becomes dysregulated and generates increasing levels of free radicals. NAD(P)H reducing equivalents may also be depleted with ramifying implications throughout the cell. Ultimately, oxidative damage leads to opening of the mitochondrial transition pore, release of cytochrome c, activation of caspases and

apoptosis. Ca^{2+} contributes to opening of the mitochondrial permeability transition pore, an action ameliorated by antioxidants [101]. Mitochondrial ion channels may be as important as uncoupling proteins in regulating membrane potential, coupling and apoptosis [94]. Ca^{2+} stimulates the tricarboxylic acid cycle and oxidative phosphorylation which increases metabolic rate and RONS generation [101].

These mechanisms are common to many forms of cellular stress and apoptosis, including excitotoxicity. In many respects they resemble smaller changes in normal signalling processes (e.g., growth factors). Excitotoxicity arises from excessive glutamate stimulation of AMPA, NMDA and kainic acid receptors with subsequent Ca^{2+} overload. Glutamate signalling, Ca^{2+} overload and "excitotoxicity" are highlighted in AD, HD, hypoxia–ischemia and PD [96, 99, 154]. RONS and elevated intracellular Ca^{2+} are reliably juxtaposed in excitotoxicity and apoptosis generally. Blockade of glutamate receptors or Ca^{2+} release receptors (IP_3R, ryanodine receptors) protects against excitotoxicity [96]. Release of zinc by mossy fibers can also exacerbate excitotoxicity via interactions with Ca^{2+}. If aged neurons have not undergone apoptosis [e.g., 38] then the processes outlined above could contribute to arrest and an inactivated state. Accumulation of an increasing subset of such cells might be expected to impact cognitive and motor performance.

Large conductance Ca^{2+}-activated K^+ channels (BK_{Ca} or Slo) are voltage-, NO-, Ca^{2+}- and redox-regulated. Specific oxidation of methionine residues increased channel activity, and H_2O_2 and cysteine-specific reagents were inhibitory [178]. Slo channels are activated by depolarization. Thus, redox regulation links channel activity, cell metabolism, electrical excitability and neurotransmitter release. Oxidation of methionine residues and channel inhibition could protect cells from excess Ca^{2+} entry during RONS stress such as associated with ischemia reperfusion [178].

Stress and apoptotic responses are linked to both Ca^{2+} and K^+ which tend to act antagonistically. Depending on their structure and function K^+ channels can either ameliorate or exacerbate apoptosis. Opening ATP-dependent K^+ channels (K_{ATP}) and Ca^{2+}-activated maxi-K channels may offset apoptosis in heart and brain. Some protective effects may be obtained by hyperpolarization, reduced membrane excitation, alterations in intracellular Ca^{2+} release and impacts on mitochondrial K_{ATP} channels. Blockade of delayed rectifier (I_K) channels was protective against hypoxia/ischemia-induced apoptosis, probably via prevention of excessive K^+ efflux [179].

Ligand-gated ion channels include the NMDA, nicotinic and purinergic P2X receptors known to impact DA neuronal survival [145]. Nicotine is depolarizing and may even protect against mitochondrial complex I inhibition by toxins and glutamate excitotoxicity [145]. Despite potential excitotoxic impacts of glutamate, moderate NMDA receptor stimulation is protective in DA neuronal culture. Elevated extracellular Mg^+ markedly decreased survival of DA neurons whereas K^+ promoted NMDA receptor activity [180]. Protection of neurons by Ca^{2+} involves a narrow and moderate elevation (35–80% over controls). Blockade of L-type Ca^{2+} channels or voltage-gate Na^+ channels decreased DA neuronal survival in midbrain slice cultures (i.e., not aged cells). Neuronal activity that activates L-type Ca^{2+} channels may promote survival via maintenance of intracellular cAMP [180].

Embryonic DA neurons were protected from spontaneous loss by a Na^+ agonist. Voltage-gated inward Na^+ channel activity was necessary to maintain protective levels of Ca^{2+} via T-type Ca^{2+} channels. Extracellular K^+ also causes a Ca^{2+} rise, probably via L-type Ca^{2+} channels [161]. Ca^{2+} activated K^+ channels (SK) modulate post-hyperpolarization amplitude and control tonic versus burst firing. Apamin, which increases neuronal activity by inhibiting SK channels also obtained protection via T-type Ca^{2+} channels [161]. Tachykinin NK_1 and NK_3 receptor activation protected mesencephalic DA neurons from spontaneous loss, acting via voltage-gated Na^+ and inward, voltage-gated N-type Ca^{2+} channels. Tachykinins are protective against excitotoxicity, β-amyloid and growth factor withdrawal [181].

Glial-derived neurotrophic factor (GDNF) induces depolarization, is strongly neuroprotective and blocks A-type K^+ channels [182]. Extracellular K^+ can also induce depolarization and protection [183]. K^+ levels sufficient to induce depolarization may only normally occur in anoxia, in which case L-type Ca^{2+} channels are involved [161, 183]. Antiapoptotic properties of extracellular K^+ can be compromised by simultaneous release of glutamate via reversed glutamate transporter function [183, 184]. This promotes excitotoxicity via NMDA and AMPA receptors in mesencephalic cultures. DA neurons were particularly impacted. K^+ channel activation remained protective if glutamate receptors were blocked. Protection likely involved L-type Ca^{2+} channels [183]. Inhibition of neuronal activity also decreased survival of neurons after axonal injury. Extracellular KCl (25 mM) which mediates depolarization, prevented cell death [185].

K^+ channel activity is associated with apoptosis in diverse tissues (neurons, cardiac cells, lymphocytes, smooth muscle, various cell cultures) [186]. K_{ATP} channel activation, hyperpolarization, loss of electrical activity and elevated RONS (e.g., via mitochondrial complex I inhibition) cause DA neuronal death, particularly in the SN. VTA neurons were not as sensitive to toxins or loss of electrical activity. Inactivation of the Kir6.2 subunit of the K_{ATP} channel was ameliorating [146]. Antioxidants were also

ameliorating reinforcing that K_{ATP} channels respond to free radicals [148, 151]. Thus, RONS- and ion-mediated DA neuronal losses are associated with electrical inactivity. Uncoupling agents that reduce mitochondrial RONS also confer protection [187].

Besides Ca^{2+} and Na^{+} influx, excitotoxic death involves massive K^{+} efflux. K^{+} efflux is an early and critical event in apoptosis including that associated with NMDA receptors. Blocking K^{+} channels or increasing extracellular K^{+} attenuates cell death, including that of midbrain DA neurons [145, 163, 188–191]. Neurodegeneration in PD corresponds to patterns of neuronal vulnerability associated with mitochondrial coupling, RONS and K_{ATP} channel activity (e.g., VTA DA neurons are less coupled and more resistant to degeneration) [145]. Here we see a nexus of free radical, ion channel and mitochondrial theories of DA aging and pathology. Further, ion-channel theories of aging highlight hypoxia and K_{ATP} channels open in response to hypoxia [148].

Increasing evidence implicates failure of protein degradation and proteosome dysfunction in aging and neurodegeneration. In rat enterocytes an apoptosis-inducing proteosome inhibitor doubled K^{+} efflux. Various K^{+} channel blockers prevented loss of mitochondrial membrane potential, cytochrome c release, caspase activation and DNA fragmentation. This reinforces K^{+} channel activity and efflux as primary apoptotic events. Voltage-gated Kv2.1 and possibly KvLQT1 channels were identified as likely mediators [186]. Apoptosis was associated with p38 MAPK activation which induces apoptosis in response to many stressors including DNA damage, osmotic stress, and RONS [186].

Kv2.1, the primary delayed rectifying K^{+} channel of neurons is the primary K^{+} efflux route during apoptosis. Oxidative stress activates the p38 pathway and p38-induced phosphorylation of Kv2.1 at serine-800. Inhibition of channel serine-800 phosphorylation blocks apoptosis [191–193]. Activation of p38 involves intracellular Zn^{2+} release via oxidative stress. Zn^{2+} release involved specific oxidation of sulfhydryl groups with subsequent p38 phosphorylation [190]. These mechanisms initiate trafficking and K^{+} channel insertion into the cell membrane. Thus, the K^{+} apoptotic surge involves recruiting channels rather than changing their properties [190, 194]. Drugs mimicking sphingosine-1-phosphate activity also induce neuronal apoptosis via a p38-caspase mechanism. This also involved an ERK-mediated reactivation of the cell cycle as evidenced by alterations in cell-cycle regulators (e.g., cyclin D1, CDK4, cyclin E and $p27^{Kip1}$) [195–198]. Re-initiation of the cell cycle (albeit abortive) may be a general aspect of neuronal apoptosis [195–197].

Release of Zn^{2+} via p38 may be mediated by NO and associated peroxinitrite. In cortical neurons NO or NMDA (which raises NO) leads to peroxinitrite formation. Peroxinitrite causes Zn^{2+} release from its main ER reservoir, the metal chelator metallothionein. Free Zn^{2+} can be taken up by mitochondria. Zn^{2+} inhibits mitochondrial respiration, induces p38, K^{+} efflux, RONS generation, permeability transition and cytochrome c release. Mitochondrial dysfunction may cause further RONS generation, formation of peroxinitrite [193] and undoubtedly, declining ATP. Antioxidants, Zn^{2+} chelators, K^{+} channel blockers and Bcl-x_L ameliorate NO-induced K^{+} efflux, neuronal shrinkage and apoptosis [193]. Pathological levels of NO are generated by excitotoxic stimulation of NMDA receptors which induces nNOS via rising intracellular Ca^{2+} levels.

Apoptosis of neocortical neurons induced by serum withdrawal or staurosporine (which causes mitochondrial cytochrome c export) was associated with massive efflux of intracellular K^{+} via delayed rectifier (I_K) channels. Extracellular K^{+} was ameliorating [199]. Although this mechanism was not observed in excitotoxic necrosis, K^{+} ionophores or channel openers also induced apoptosis. Cell shrinkage associated with rapid K^{+} efflux is reliably associated with apoptosis. Remarkably, the outward K^{+} current is induced by cytochrome c, the primary mediator of apoptosis released from mitochondria [200]. Bcl-2 overexpression in vascular smooth muscle cells decreased K^{+} channel activity and K^{+} export. Transcription of Kv1.1, Kv1.5 and Kv2.1 were downregulated. Thus, inhibition of K^{+} channel activity is a key anti-apoptotic mechanism associated with bcl-2 [201]. K^{+} channel activity fundamentally contributes to aspects of cell survival and apoptosis. There may be several pathways to K^{+} export since it appears to be a very early event in some circumstances that might be expected to precede cytochrome c release.

Chronic depolarization mediated by high levels of extracellular K^{+} protected embryonic rat neurons from growth factor withdrawal although this also inhibited growth (e.g., neurite extension). This was associated with Ca^{2+} influx, probably via L-type channels, although protection did not closely correlate with Ca^{2+} levels [202]. This suggested a threshold response to Ca^{2+}, perhaps associated with the degree of binding to calmodulin [202]. Alternatively, activation of K^{+} channels and efflux (especially kv2.1, ATP- and RONS-sensitive K^{+} channels) may be more critical than intracellular Ca^{2+} for neuronal apoptosis. High extracellular K^{+} may be protective [185].

Calmodulin

Ca^{2+} activates NFκB and calmodulin-CREB which can be neuroprotective [96]. Calmodulin is inhibited by reversible oxidation of key methionine residues. Methionine oxidation likely reduces Ca^{2+} signaling associated with oxidative conditions, including aging. Inactivated

calmodulin accumulates in aged cells, but may be reactivated by methionine sulfoxide reductase. RONS slow down inactivation of a voltage-dependent K^+ channel via interaction with a specific methionine residue, and this can be reversed via methionine sulfoxide reductase [203]. Calmodulin has >50 regulatory targets including Ca^{2+} pumps, ion channels, NOS and antioxidants [202, 204]. The inactive protein still binds and stabilizes target molecules. Despite a relatively short half life of ~18 h (oxidized calmodulin is degraded by the proteosome) at least some calmodulin methionine residues are oxidized in senescent rat brains. Virtually all calmodulin is oxidized in skeletal muscle whereas little is oxidized in heart [204].

Cellular Ca^{2+} gradients can achieve a 10,000-fold concentration differential. Maintenance via Ca^{2+} pumps and the Na/Ca^{2+} exchanger are major energetic sinks for ATP. The Ca^{2+} regulatory proteins calmodulin and Ca-ATPase are exceptionally sensitive to oxidation and nitration at specific sites. This could prove adaptive by reducing cellular ATP consumption and RONS under oxidative stress [205] but may be distorted by chronic oxidative stress in aging. A further target of calmodulin is NOS. Reduced respiration and ATP production would also allow available reducing equivalents to be sequestered towards antioxidant defences. Reduction of oxidized cysteine residues can be achieved via reducing equivalents supplied by GSH and thioredoxin. Reversal of methionine oxidation can be mediated by thioredoxin and methionine sulfoxide reductase. All depend on NADPH reducing equivalents. Reduced calmodulin expression in aging could up-regulate stress response elements and decrease metabolism [205].

Channels and Electroplasmic Cycles

Ion channel and redox regulation appear virtually inseparable. Cellular features regulated by ion channels include cell size, osmotic pressure, pH, nutrient uptake (including amino acids), neuropeptide secretion and reuptake, excretion, detoxification, muscle contraction, mitochondrial function, growth and apoptosis. For neurons, channels are critically involved in electrical activity. Thus, linking redox and RONS to ion channels aligns an immense amount of cellular functions. If we extend redox even further to clock and sleep/wake functions we can add numerous other processes to forge a truly global framework as outlined below. Indeed channels are controlled by the clock [206].

The Mammalian Regulatory Bauplan: It's about Time

The "*Free Radical Theory of Aging*" holds that RONS cause aging via accumulating damage to DNA, proteins, lipids, and higher-order structure [110, 207, 208]. RONS are implicated in cognitive aging and most neuropathologies, including PD. Dysregulation of aging mitochondria and the ubiquitin-proteasome system are critical [7, 111, 209, 210]. RONS can inhibit neuronal activity and neurotransmitter release and ultimately cause apoptosis. DA systems, particularly SN are exceptionally sensitive to RONS and excitotoxicity [10, 107, 118, 149–151, 211].

The free radical theory expanded recently from a focus on damage to recognize pervasive roles for RONS in cell signalling and diverse functions, including neuronal activity and synaptic plasticity [e.g., 103, 212–215]. Thus, SOR associated with NMDA receptors is required to activate PKC and MAPK-ERK involved in LTP [118, 133, 216, 217]. Thus, age-related RONS imbalance may derive widespread signalling distortions.

Redox state extensively regulates cellular functions via reversible modifications of protein cysteine and methionine residues. This involves glutathione, thioredoxin, glutaredoxin, nitric oxide synthase, NOX and even mitochondria [84, 178, 213, 218, 219]. Oxidative inhibition of tyrosine protein phosphatases brings cell transduction networks regulated by kinase-phosphatase antagonism under redox control [220–222]. Key aspects regulated via cysteine or methionine switches include DA functions (TH, DAT, DJ-1, ion channels), antioxidant and stress responses (Ref1, Nrf2, Keap1), heat shock and chaperone proteins, ion channels (calmodulin), apoptosis (caspase 3), cell cycle, metabolism, mitochondria, detoxification, cell signalling (cytokine and growth factors), and protein trafficking and degradation (proteasome and ubiquitin-associated factors). DNA binding by transcription factors is also regulated by redox-sensitive cysteine residues (e.g., AP-1, NFκB, nuclear factor-1, Sp-1, HIF-1α and p53) [94, 103, 152, 203, 213–215, 218, 219, 221, 223–226]. All this highlights redox and RONS as *primary* determinants of cell functioning.

We proposed an "Allocative Theory of Sleep" suggesting that wake-sleep cycles represent temporal compartmentalization of functions, with niche-interfacing activities (athletics, perception, thermoregulation) relegated to waking and growth, housekeeping, restorative and immunological processes restricted to sleep. Restricting anabolic functions to sleep maximizes resources for niche interfacing (e.g., foraging, avoiding enemies, acquiring mates). The HPA globally regulates waking, and the antagonistic GH axis regulates sleep functions [65, 227–229]. Temporal sequences of behaviour and associated functions reflect an underlying march of regulatory neuropeptides, with DA linked to the HPA, arousal, motivation and motor activity. Thus, this framework encompasses temporal regulatory organization spanning neuroendocrinology, neurotransmitter activity, cell transduction networks and gene transcription. For mammals this largely

involves ultradian (3–4 h), circadian (24 h) and circannual (~1 year) rhythms.

This framework recently exploded with the fusion of endogenous clocks to redox regulation. Thus, the yeast respiratory cycle is proposed to function in temporal compartmentalization of various key functions [230, 231]. Fusion of clocks and redox adds temporal coordination to redox-regulated functions (e.g., signalling networks, neuropeptide release, chromatin structure, gene transcription, amino acid metabolism, protein synthesis, protein degradation, cell cycle, transport (especially cytosolic-nuclear), chaperones, ion channels, glucose and lipid metabolism, heme synthesis, replenishment of antioxidants and cellular reducing equivalents). Thus, a fundamental regulatory bauplan (blueprint) directing temporal sequencing, integration and compartmentalization of most cellular functions emerges. Electrical properties are particularly emphasized for neurons. Thus, this dynamic organization might be described as an "Electroplasmic Cycle" [103].

The master mammalian clock resides in paired suprachiasmatic nuclei (SCN) in the hypothalamus (the microprocessor coordinating allocation of resources and energy to various functions). However, all tissues express endogenous clocks. Glutamatergic projections from the retina to the SCN provide photic input that maintains phase with day-night cycles. The circadian clock directly regulates waking and activation of the HPA. Thus, CLOCK directly controls arginine vasopressin transcription. This in turn mediates output from the SCN to targets such as the paraventricular nucleus and orexin system that modulate the HPA and arousal [232, 233]. Sleep is sensitive to the clock (and HPA) but it is flexible. Fitness requires foregoing sleep to meet extended waking needs or to awaken if a bear invades your tent. Thus, the HPA has eminent regulatory domain and gene arrays distinguish clock-regulated genes from those associated with arousal states. Sleep is homeostatic, however, and sleep deprivation is ultimately lethal.

The circadian clock employs diverse regulatory mechanisms but the core involves transcription of two clock effectors, *Cryptochrome* (*Cry*) and *Period* (*Per*) by dimers of the gene products of *Clock* and *Bmal1*. Translated CRY-PER dimers translocate to the nucleus and inhibit transcription of their own genes, thus creating a negative feedback loop and timing function [234]. Neuronal PAS domain protein 2 (NPAS2) is an analog of CLOCK expressed in brain. DNA binding by CLOCK:-BMAL1 and NPAS2:BMAL1 heterodimers is regulated by redox status of the nicotinamide adenine dinucleotide cofactors, NAD(H) and NADP(H). NADH and NADPH (reduced forms) enhance dimerization and DNA binding whereas oxidized forms are inhibitory. Thus, NAD(P)H/NAD(P) balance regulates CLOCK [213, 220, 235, 236]. CLOCK and NPAS2 may function as redox sensors [220]. NPAS2 binds heme to create a sensor for CO. CO inhibited DNA binding of heme-loaded NPAS2 and favoured formation of inactive BMAL1 homodimers over active NPAS2/BMAL1 heterodimers [235].

Metabolism, circadian rhythms and redox are linked [236]. Food (energy levels) impacts the clock supporting a linkage to energy metabolism. One target of NPAS2:BMAL1 is lactate dehydrogenase which reversibly derives lactate from pyruvate. This consumes NADH and generates NAD. Production of reducing equivalents of NAD(P)H was highlighted as a key function of the yeast reductive phase and NAD(P)H/NAD(P) strongly cycled [231]. Cycling of glucose-6-phosphate 1-dehydrogenase (and consequent rhythms of NAD(P)H production) represents an important regulatory loop for the *Drosophila* clock [237].

Any doubt that vertebrate clocks are inseparably linked to redox is dispelled by results with Z3 cells from zebrafish embryos that entrain to light in cell culture [238]. Remarkably, light induction of H_2O_2 mediates photic signalling to the endogenous circadian clock. H_2O_2 induced zCry1a and zPer2 and circadian oscillations in zPer1. H_2O_2 itself mimicks effects of light [239]. Catalase oscillates in opposite phase to zCry1a and zPer2 and catalase modulates sensitivity of these core clock genes to light [239].

Heme subserves metabolic enzymes and transcriptional regulators of circadian rhythmicity. Heme functions in O_2 transport, detoxification, signal transduction and as a prosthetic group to metabolic enzymes. In yeast, aminolevulinic acid (a rate limiting element for heme biosynthesis) rose at the end of the oxidative phase. Reducing conditions may be necessary to regenerate heme prosthetic groups of cytochromes following intense respiration [231]. Heme oxygenase (HO), which breaks down heme, peaked in the late charging phase. Heme biosynthesis emerges as strongly circadian in mammalian gene arrays. NPAS2 regulates expression of aminolevulinate synthase 1 (Alas1). Heme enhanced expression of mPer1 but inhibited mPer2 via a mechanism involving NPAS2 and mPER2. Both NPAS2 and mPER2 have heme-binding PAS domains. Vitamin B12 competes for PAS binding, antagonizing impacts of heme on *mPer1* and *mPer2* expression [239].

The SCN strongly regulates circadian rhythms of glucose in rats, which peak just prior to activity. This coincides with circadian rhythms in glucose uptake and insulin sensitivity that are ablated by SCN lesions [240]. Gene arrays confirm circadian rhythms in glucose and lipid metabolism, ATP production, and heme biosynthesis [241]. Heme reversibly binds REV-erbα and modulates its interaction with a nuclear repressor complex. In turn, REV-erbα represses hepatic genes regulating gluconeogenesis and glucose export. Thus, the heme-REV-erbα

interaction links the clock to hepatic glucose production and global energy metabolism [241]. Synthetic and catabolic heme pathways can lead to toxicity. HOs are protective and their products (biliverdin and bilirubin) are antioxidants. Offsetting this is the release of iron from oxidized heme which can contribute to hydroxyl radical formation [242]. Elevated HO-1 was detected in neuromelanin neurons in the SN of PD victims in close association with Lewy bodies. Only the SN was so afflicted, suggestive of exceptional oxidative stress. Excessive heme-derived iron, and HO-1 associated CO generation would elevate neuronal stress [11].

The targeting of a histone deacetylase complex by REV-erbα to the *Bmal1* gene [241] is significant given that *Clock* encodes a novel histone acetyltransferase, and BMAL1 enhances acetyltransferase activity. Other acetyltransferases (e.g., p300, ACTR, CBP, PCAF) also recruit to CLOCK:BMAL1 complexes [234, 243]. CLOCK/BMAL1 heterodimers stimulate transcription of *Cry* and *Per* via acetylation of H3 histone proteins, and alterations in RNA polymerase II binding [243]. Histone deacetylase 1 also shows cyclical expression in coordinated fibroblasts [223].

Cyanobacterial rhythms of circadian transcription involve global shifts in the degree of chromosomal supercoiling, suggesting an ancient linkage of time keeping and DNA conformation [244]. This suggests that rhythms of acetyltransferase versus deacetylase antagonism may derive rhythmic shifts between euchromatin and heterochromatin (and euchromatin would be more susceptible to DNA damage). Although many specific regulatory programs involve targeted alterations in chromatin structure (e.g., Sirtuins and the dietary restriction response) heterochromatin granules in human buccal epithelium showed a circadian rhythm with greater condensation at night and less in morning (following antioxidant recharging?). Physical activity caused condensation, likely mediated by catecholamines and cortisol [245].

How clocks impact aging remains unclear [246] but a balance between oxidative processes and reductive protection, detoxification, recharging and restoration seem likely. Coordination of chromatin structure with appropriate redox states may protect DNA from oxidation. Although longevity extensions are reliably obtained by sirtuin deacetylases [247] longevity of *Drosophila* was also extended by ~40–50% by pharmacological inhibition of deacetylases [248]. Increased longevity did not alter locomotion, reproduction or stress resistance. Histone acetylation was globally elevated and gene expression was complexly altered (including increased superoxide dismutase (*SOD*), glutathione S-transferase (*GSH-S-TR*), Cytochrome P450, and 3 chaperones). Downregulated genes included glyceraldehyde-3-phosphate dehydrogenase, NADH ubiquinone reductase, cytochrome oxidase subunit VIb, fatty acid synthetase and cyclin-dependent kinase [248].

Extension of longevity may require optimal balance between expression and repression of alternative gene sets regulated by changes in chromatin structure [248]. Droge [215] argued that interventions to extend longevity should not compromise wake-associated activity and brain functions. Interestingly, *Sir2/Sirt1* deacetylase is NAD^+-dependent [247] providing indirect clock linkage. *Sir2* is functionally linked to *Sas2*, a histone acetyltransferase related to *Clock* [234].

NAD(P)H Oxidases: Rhythmic Mediators of Signalling and Redox Status?

NAD(P)H oxidases (NOX) contribute to numerous pathologies (including PD) and aging itself. NOX occur on both neurons and glia in brain [26, 177, 212, 249–253]. Angiotensin II, which activates NOX via AT_{r1}, exacerbated DA neuronal losses induced by 6-hydroxydopamine in rats. NOX inhibition was protective suggesting that angiotensin and NOX expression contribute to stress in DA systems [254]. NOX impact GSH and redox-sensitive cysteine status [177, 255, 256]. NOX alternate between generating superoxide radical and performing protein-disulfide-thiol interchange with a period of ~24 min. The protein disulfide-thiol interchange period drives cellular enlargement. Activities involving cysteine residue modifications by NOX suggest a tight linkage to redox-mediated cellular signalling and the GSH system. NOX isoforms represent the terminal oxidase in a system that transfers electrons from cytosolic NAD(P)H through the cell membrane [257]. Membrane CoQ10 may be a necessary substrate. This indirectly links NOX function to that of mitochondria [152].

Plasma membrane redox systems can modify cytoplasmic NAD(P)H/NAD(P) ratios see [152, 255, 258]. NAD(P)H/NAD(P) status impacts numerous cell functions (e.g., ATP production, PARP and DNA repair, GSH and DA synthesis and the clock). NAD(P)H is also a critical intra-cellular antioxidant [259]. Activity of membrane redox systems may increase NAD^+, thereby stimulating sirtuins (SIRT) and inducing a dietary restriction response that slows aging.

Circadian clocks coordinate activities of differentiated cells and also exerts cell-cycle control [260]. In this regard activation of growth factor receptors (and some neurotransmitters) generates free radicals via membrane-bound NOX which is essential for MAPK-ERK signalling [64, 212, 261, 262]. Radiation alone can activate growth factor pathways and elicit growth [64]. Many CLOCK-controlled genes function in the cell cycle and the cell cycle is inhibited in *Clock* mutant mice [263]. This involves both

downregulation of genes and overexpression of inhibitors such as $p27^{Kip}$ and $p21^{Waf1}$.

Down-regulation of CLOCK-controlled genes in mutant mice illustrates that a robust clock may be required to obtain youthful patterns and levels of gene expression for youthful functioning [264]. The cell cycle links to redox via cysteine switches in many regulatory peptides [219, 265]. Besides functioning with growth factor receptors NOX inhibition arrests proliferating fibroblasts at the G_1 checkpoint and to a lesser degree at G_2 [266]. Radiation compensated for decreased RONS and attenuated G_1 delay.

G_1 delay induced by NOX inhibition increased p53 activity and decreased cyclin D1, ERK 1/2 and p38 activity. Using mutant ATM or $p21^{Waf1}$ cells, it was shown that G_1 delay was controlled by both. ATM regulation was sensitive to free radical processes independently of DNA damage. NOX activity was greatly increased in cells with dysfunctional ATM or $p21^{Waf1}$ suggesting a regulatory connection. Alternatively, NOX-induced free radical generation and redox shifts likely modulate G_1 checkpoint regulators [219]. Notably, inhibition of NOX was associated with increased levels of p53 that traced to inhibition of the proteasome and proteolysis [266]. Proteolysis mediated by the proteasome particularly regulates the cell cycle, and accumulation of dysfunctional or aberrant proteins with age likely reflects oxidative inhibition of proteasome function.

The alternating functions of NOX represent an ultradian clock entrainable and responsive to melatonin and blue light [256]. Remarkably, transfecting cells with NOX isoforms with period lengths spanning 22–42 min, yielded rhythmicity in the clock-controlled protein glyceraldehyde-3-phosphate dehydrogenase with periods of 22–42 h [255]. This strongly suggests hierarchical compounding of cycles occurs across ultradian periods of ~24 min to circadian rhythms of 24 h. Responsiveness to melatonin supports the hypothesis that cell cultures might be entrained by melatonin pulses. Many features of NOX suggest that they represent a component or extension of clocks (e.g., entrainable oscillator, RONS-generation, NAD(P)H utilization, protein disulfide-thiol interchange). O_2 consumption may also fluctuate with NOX activity [257]. NOX-mediated RONS generation and linkage to cytosolic NAD(P)H provides a mechanism capable of toggling cells between redox states [see 152].

Because NOX generate and are activated by free radicals, they can become self-sustaining [251]. NOX may potentially synchronize intercellular redox dynamics or transmit free radical stress signals. The association of NOX with numerous pathologies, including PD, makes NOX a target for therapeutic interventions [267]. Although NOX generation of free radicals may impact the residing cell in many cases RONS may be compartmentalized (receptor function, immunological phagocytosis) or directed extracellularly. This could manage intracellular redox or mitochondrial function. If so, inhibiting NOX might amount to plugging the exhaust pipe on a car that is burning oil.

What is Cycling?

Clocks arose in unicells and vertebrates express secondary neuroendocrine overlays and structure dedicated to temporal organization (e.g., retinohypothalamic tract, SCN, pineal gland, HPA-GH axes antagonism, sleep-wake regulation). Circadian rhythms occur in transcription, mRNA stability, translation, transport, protein degradation and higher-order functions. Translational control is highlighted in dinoflagellate algae whereas rhythms of genome-wide transcription occur in cyanobacteria [244]. For the hepatic "proteome" ~20% of soluble proteins are circadian even if genes are not [268]. In most organisms or tissues ~1–10% of genes show circadian expression [223, 269, 270]. Rhythmicity of specific genes is highly variable and tissue-specific such that most genes likely cycle in one tissue or another [270].

Cell cultures can be synchronized by numerous factors including serum shock [223, 271]. The pineal hormone melatonin coveys a global timing signal in vivo. Clock genes and their targets contain E-Box (or Clock) consensus sequences. Circadian transcripts particularly regulate metabolism, tissue homeostasis, cell cycle, DNA synthesis, detoxification, biosynthesis, neuropeptides and immunity [272, 273]. GH strongly regulates the immune system and sleep can be induced by substances derived from parasites, bacteria and viruses. Sleep deprived animals usually die of infections. Ablation of the SCN dampens temporal gene expression in peripheral tissues [269].

The greatest advances in understanding the coordination of functions by redox and clocks comes from unicells (e.g., *Saccharomyces cerevisiae*). At sufficient densities or under nutrient limitation yeast express synchronized ultradian rhythms of respiration and glycolysis regardless of whether grow occurs see [231, 274–278]. More than half of the genome cycles and subsets of genes exhibit function-specific co-expression. Genes contributing to metabolism and energetics are particularly rhythmic. Cycles in gene expression, cell cycle and glucose metabolism were closely coupled and >25% of genes correlate with growth rate. Peroxisomal functions negatively correlated to growth whereas ribosomal and stress response genes positively correlated to growth [278].

Cycle lengths of yeast and other unicells vary from ultradian rhythms of 40–50 min, 4–5 h, or circadian rhythms of ~24 h [e.g., 222, 231, 270, 274, 275, 279]. Regardless, the relative structure of sequential functioning

remains similar across various periods. Yeast ultradian rhythms reflect a temperature-compensated clock that may be an archetype for clocks of greater period [222]. Klevecz et al. [274] found >87% of genes expressed in the reductive phase (4,679 transcripts) but a small peak also occurred in the oxidative phase (650 genes, ~12%). Expression in the reduced phase was bimodal with superclusters of nearly equal size occurring in the early and later phases.

These three peaks in gene transcription likely relate to three functional states that Tu et al. [231] identified across yeast metabolic cycles: 1) oxidative, 2) reductive-building and 3) reductive-charging. A profile of 130 key metabolites revealed 40 with robust rhythmicity (including amino acids, GSH/GSSG and NADP(H)). The oxidative phase was largely dedicated to respiration and energy (ATP) production. The reductive/building phase represented a shift to glycolytic metabolism and reduced respiration. Production of acetyl-CoA and NADPH were enhanced during the reductive/charging phase. The main purpose of the pentose pathway appeared to be NADPH production (rather than pentose sugars). The coordinated inter-regulation of the cell cycle and circadian and metabolic rhythms with transcription and DNA replication restricted to reducing conditions may protect DNA [231, 274, 275].

Remarkably, disruption of the pentose pathway ablated metabolic rhythmicity, suggesting a key role of NADPH in the cycle. NADPH is the major source of reducing equivalents, and the NADPH/NADP ratio is maintained in a state biased to reduction (~20:1 or greater). A critical function of the reduced phase in yeast is replenishment of NAD(P)H reducing equivalents and reduction of the glutathione antioxidant system to support functions during the oxidative phase. Amino acids dependent on NADPH as a substrate showed peak synthesis in the oxidative phase whereas others (e.g., glutamate, aspartate, asparagine) were gated to the reductive-charging phase. In rats, 5–10 d of sleep deprivation reduced hepatic catalase and GSH by 23–36% [280]. Liver exports antioxidants that serve as an extracellular buffer so large changes in hepatic antioxidant status are of great significance. Thus, both sleep and clocks are intermeshed to redox.

As O_2 levels decline and H_2S levels rise, DNA replication ensues in growing yeast cultures. H_2S is of great significance and is associated with a sulfur uptake and utilization cycle involving cysteine and methionine (multiplicatively linked to redox signalling and control). Sulfur metabolites (including cystathionine, homocysteine, homoserine, serine, S-adenosylhomocysteine (SAM) and GSH) are highly cyclic in yeast and contribute to metabolic cycling itself. Synthesis of sulfur-containing methionine and cysteine requires four NADPH reducing equivalents to reduce sulfate to sulfide. Sulfide is then used to synthesize homocysteine. This in turn contributes to production of cysteine (and GSH) in one pathway, or methionine and SAM via another (potentially competing) pathway [231]. During the reductive building phase sulfur metabolites are directed away from GSH and into SAM synthesis, supporting methylation of new histones, DNA, mRNA and other macromolecules associated with cell division. SAM synthase expression peaks in the late oxidative phase whereas GSH synthase peaks later in the reductive building phase [231].

Rhythmic aspects of yeast included redox, sulfate uptake, ethanol production, the NAD(P)H/NAD(P) redox couple, GSH/GSSG antioxidant status, and pH [281, 282]. Mammalian cell cycle progression is also associated with GSH status. Levels of NAD(P)H (mainly NADH) peaked as dissolved O_2 rose (respiration declined) and injections of GSH or GSSG altered the rhythm and inhibited respiration [270, 283]. GSSG also strongly induces sleep in vertebrates [284] and GSSG elicited greater phase shifts in yeast [283]. Metabolic synchronization was associated with peaks of H_2S release in opposite phase to those of acetaldehyde. H_2S rose sharply as respiratory activity declined. These materials likely synchronize cells in culture. Mitochondria showed cycles of energization, membrane potential and ultrastructure [281, 282].

The pentose-phosphate pathway converts NAD(P) to NAD(P)H. Glucose-6-phosphate 1-dehydrogenase gates entry to this pathway, and in *Drosophila*, gene activity is maximal toward the end of the day. Opposing this activity and mediating gluconeogenesis is fructose biphosphatase which peaks at dawn. Thus, antagonistic pathways drive circadian rhythms of glucose anabolism and catabolism [237, 270]. Pathways for sugar and urea show similarly circadian organization in mouse liver [268].

The metabolome of yeast cycles with 70% of elements in phase with NAD(P)H peaks. A transcriptional complex contributing to biosynthetic, reductive and cell cycle functions is responsive to TOR (which senses amino acid status of the cell). Rapamycin induced the reductive phase for 60 h. A distributed network of interacting elements rather than a central regulatory oscillator may derive yeast cycles [222, 285]. Interestingly, MAO-A inhibitors also alter the period of the yeast rhythm [286].

Avoidance of UV exposure may have restricted the S-phase to the dark to avoid DNA damage in ancient cells. *Per-1* entirely localizes to the nucleus following irradiation suggesting a redox-sensitive localization signal [260]. γ-Irradiation induces numerous clock genes in liver (*mPer1, mPer2, Clock, Cyr1, Bmal1*). Mice lacking *mPer2* function are radiosensitive and tumor prone [287]. These mice lack oscillations in *c-myc* expression which could dysregulate p53. Remarkably, PER1 associates with ATM and CHK2 [260]. Overexpression of *Per1* increased apoptosis of cancer cells in response to radiation. This

involved upregulated *c-Myc* and attenuation of $p21^{Waf1/Cip1}$. Inhibition of *Per1* suppresses apoptosis and *Per1* expression is reduced in cancer [260].

CLOCK:BMAL1 regulates c-MYC, which cooperates with ATM to regulate apoptosis and inhibit tumorigenesis [260]. In *Drosophila*, the clock gene *Tim* (which heterodimerizes with PER) associates with ATM-related kinase to regulate the cell cycle. This links the clock to DNA repair and cell cycle checkpoints [273]. Moreover, *mPer2* is coupled to cell cycle regulators including *Cyclin D1, Cyclin A, Mdm-2, ATM, Chk2* and *GADD45a* [273] suggesting relevance to cancer.

In cyanobacteria photosynthesis and nitrogenase activity (nitrogen fixation) are temporally compartmentalized to opposite phases. Thus, oxygen-sensitive nitrogenase is protected from oxygen-associated photosynthesis [244]. Tu et al. [231] make a similar case for restriction of several other functions to reducing conditions, including oxidation-sensitive heme metabolism, sulfur metabolism, accumulation of NAD(P)H reducing equivalents and recharging pools of antioxidants like GSH. In terms of electroplasmic cycles, this is equivalent to recharging the battery.

Clock Genes and Brains

Cycling genes in *Drosophila* are under the control of Clk. In the head, circadian genes regulated perception, learning, signal transduction and synaptic function (including vesicle transport), vision, olfaction, and locomotion. Protein processing also showed circadian rhythmicity (protein stability, degradation, proteasome function and ubiquitin-related enzymes). Heme metabolism (δ-aminolevulinate synthase, and heme oxygenase) were highlighted. Because heme chelates iron, dysregulation of synthetic-degradation pathways could exacerbate production of hydroxyl radical [206]. Heme is required by many P450 enzymes involved in steroid synthesis and detoxification.

Detoxification and stress-response genes were highly rhythmic (including P450s, catalase, tocopherol and GSH-S-TR and thioredoxin) [206, 237]. Clock control of tocopherol-binding proteins, tyrosine 3-monooxygenase, and glucose-6 phosphate 1-dehydrogenase was of interest. The K^+ channel gene, slowpoke, was associated with core clock genes. Clock control of the slob Ca^{2+} binding protein (that interacts with slowpoke) suggested circadian impacts on membrane potential and neuronal functioning via K^+ channels [288]. At least twelve genes suggested sleep-associated detoxification [237].

Of 15,000 genes expressed in rat cerebral cortex $\sim$5% were associated with wake or sleep states. Wake-associated genes functioned in metabolism, neurotransmission, transcriptional activation, and cellular stress responses. Sleeping was associated with myelin formation, synaptic vesicle turnover, synaptic consolidation and protein synthesis [171]. Of 2,032 cycling genes in mouse brain, 391 were circadian and the rest were sleep-wake dependent [289]. Overexpression of several genes (especially Homer1) was correlated with sleep deprivation. These genes function in recovery from excitatory glutamate signalling and associated Ca^{2+} alterations supporting a strong role of brain recovery for sleep [289]. A role of sleep in recovery from excitotoxicity is particularly relevant to DA, and the stress of sleep deprivation is highlighted. Sleep was associated with expression of 2,090 genes in mouse cerebral cortex and 409 in hypothalamus [290]. The largest functional categories were biosynthetic (e.g., cholesterol, heme, antioxidants, metabolic proteins), transport, and maintenance of vesicle pools [290].

In avian telencephalon waking genes were involved in energy metabolism, oxidative phosphorylation, activity-dependent neural plasticity, and stress responses (heat shock/chaperone proteins). Sleep gene functions included membrane trafficking, lipid/cholesterol synthesis, protein synthesis, cellular adhesion and cytoskeletal organization. Others of interest included K^+ channel regulators (e.g., kir3.1, KV 9.1, kv5.1), vasoactive intestinal peptide, histone deacetylase 9 and glial fibrillary acidic protein. Sleep may be particularly associated with synaptic downregulation, remodelling and neuronal vesicular protein traffic [291]. The latter is highlighted in DA oxidation.

Dopamine, Clocks and Sleep

DA bursts from the SN to striatal spiny neurons provide central pacemaker function and interval timing abilities on scales of milliseconds to minutes. In PD striatal timers run slow [292]. Other rhythms include ultradian cycles that vary in minutes to hours, circadian rhythms of 24 h and circannual rhythms of about one year. Many temporal cycles are hierarchically compounding such that ultradian rhythms of rodents (3–4 h cycles of waking-grooming-foraging-feeding and sleep) contribute to similar patterns emerging on a circadian basis. Profoundly, redox lies at the core of temporal organization as reflected by NAD(P)H/NAD(P) and GSH/GSSG redox couples. This extends to rhythmic oxidation-reduction, glutathionation and nitration of sensitive cysteine and methionine residues that constitute redox switches.

DA cell groups may regulate sleep, motivation and motor activity. The role of DA in sleep control has been obscure for mammals but emerging evidence implicates the VTA. VTA DA neurons are excited by orexin (OX). Sleep-state dependent DA neurons occur in the periaqueductal gray which is a VTA extension [293]. Expression of the catabolic catecholamine enzyme arylsulfotransferase is proportional to length of sleep deprivation and may

function to interrupt brain catecholamines in sleep induction [294]. The mesocorticolimbic DA system regulates thalamocortical arousal state associated with sleep-wake regulation. Orexins are also clearly involved here [295–298]. Low levels of DA may induce sleepiness via feedback to DA_{r2} autoreceptors. Levels that stimulate locomotion inhibit sleep and promote arousal via DA_{r1} mechanisms [299].

Although DA is not directly involved in adult SCN regulation, the developing mammalian fetus expresses DA_{r1} mRNA in the SCN, which provides the signalling conduit for maternal entrainment of the fetal clock. Following birth this is replaced by photic signals from the retina to the SCN via NMDA receptors [300]. Although direct DA control of the SCN is lost in adults a critical role of DA in retina is phylogenetically conserved. Some invertebrates and all vertebrate phyla have retinal circadian clocks and for some these are the dominant timekeeper. In mammals, photic signals to the SCN are integrated in retinal ganglion cells.

Retinal clock function involves antagonistic interactions of melatonin (structurally related to serotonin) and DA. DA peaks in the light period and sensitizes cones, whereas melatonin reigns in the dark and sensitizes rods. DA regulates light adaptation and rhythmic expression of melanopsin found in light-sensitive retinal ganglia cells [189, 301–308]. Light pulses raise DA levels, inhibit melatonin and phase shift the retinal clock. Alternatively, DA itself mediates phase shifts. DA and melatonin have antagonistic impacts on one another if administered at various times of day. TH has a retinal circadian rhythm with threefold higher expression in daytime.

Shifts between rod- and cone-dominant states are mediated by DA_{r2} (with activation favoring cones) (see [189, 302–308]). DA links retinal photic input and cell signalling to circadian clock elements via DA_{r2}. This involves activation of MAPK-ERK and the acetyltransferase CREB binding protein (CBP). DA_{r2} signalling enhances transcription of mPer1 by CLOCK:BMAL1 heterodimers via recruitment of CBP. Thus, DA_{r2} null mice have reduced photic induction of *mPer1* [309]. DARPP-32 is an important target of DA signalling that regulates transcription factors, voltage-gated ion channels and neurotransmitter receptors. In DARPP-32 knockout mice, light exposure failed to fully obtain a normal behavioral phase delay or induction of *mPer2* in the SCN. DARPP-32 expression was found in retina but not the SCN suggesting a retinal clock or signal transmission function [310].

Remarkably, retinal dysregulation occurs in PD. This manifests in reduced light sensitivity, light–dark adaptation, contrast perception, and loss of color vision (blue sensitive B cones) largely associated with losses of DA. When normal ageing is associated with retinal degeneration this appears similar to PD but loss of DA is less [311]. L-DOPA significantly improved color vision in PD [312]. Deterioration of color vision in PD increases with disease progression [313]. Depletion of retinal DA with 6-OHDA produced functional and morphological changes in quail retina resembling old age. Normal age-related loss of DA in regions of the retina ranged as high as 50% [314]. In senile rats (24 months old) deficits in retinal DA were associated with visual deterioration [315]. Increases in activated microglia in retina occurred in aging pigeons and quail and numbers were inversely related to photoreceptor number [316].

Regulation of DA by the Clock

DA is strongly regulated by the clock. In a 12:12 L:D cycle DA, DOPAC and HVA showed parallel circadian rhythms in striatum of awake rats. All peaked in mid-dark (peak rat activity period) and expressed nadirs in mid-light [317]. Mice lacking *Clock* displayed increased DA activity, enhanced locomotion and increased reward from cocaine in the VTA. This was associated with elevated expression and phosphorylation of several DA-associated genes, including TH and a voltage-gated K^+ channel [318].

Locomotion is a robust biomarker of clock function and DA regulates arousal in *Drosophila* [172, 319]. The *Drosophila* homolog of TH (Ple) demonstrates strong circadian cycling which contributes to circadian rhythms of neurotransmission and locomotion [206]. Ebony, a β-alanyl DA synthetase associated with levels of DA, contributes to locomotor rhythmicity in *Drosophila* [237]. In *Drosophila*, DA_{r2} responsiveness to quinpirole (induced locomotion) shows circadian rhythmicity. The circadian rhythm in DA responsiveness required normal PER function in peripheral tissue [320]. *Drosophila* expressing mutant or normal forms of α-synuclein had adult-onset loss of DA neurons, neuronal inclusions containing α-synuclein and dysregulated locomotion. Remarkably, given the retinal DA loss in mammalian PD, expression of α-synuclein caused progressive retinal degeneration in flies [321].

The Aging Clock

Disruption of circadian rhythmicity decreased longevity of the hamster *Mesocricetus auratus*, whereas SCN implants restored high-amplitude activity rhythms and extended lifespan [322]. $Tau^{(+/-)}$ hamsters had ~20% shorter lives than either homozygote but others found that homozygous $Tau^{(-/-)}$ mutants lived ~15% longer even though their metabolic rate was ~20% higher [323]. Typically, imminent death was associated with arrhythmia. Acceleration of season photoperiodic cycles causes accelerated aging in mouse lemurs, including early aging of the clock itself

[324]. αMUPA transgenic mice showed increased longevity associated with robust circadian rhythmicity, and in liver, high expression of *mPer1*, *mPer2*, *mClock* and *mCry1* (but not *Bmal1*) [264]. The authors suggest that life extension via dietary restriction involves robust rhythmicity and strong functional synchronization.

Bmal1 and *Per* particularly influence aging [244]. Shift work is associated with increased risks of cardiovascular disease, diabetes and cancer. Aging clocks are associated with reduced amplitude in numerous functions including hormonal rhythms, core temperature and sleep-wake cycles. The SCN shows altered function, neuronal loss and reduced photic input—see above [325]. *Clock* and *Bmal1* particularly exhibit reduced age-related expression (and these are the clock genes most affecting aging). *Per1* mutant mice are more susceptible to radiation and some consequences resemble accelerated aging [246]. Weekly phase-shifting of the clock has little impact on young mice but increased mortality in old mice (27–31 months) [326]. Old mice experiencing phase advances for 8 wk suffered 53% mortality whereas phase delays yielded 32% mortality. Only 17% of unshifted mice died [326]. Protein carbonyls were 30% higher in mutant Per^S flies (which have a shortened period). Per^0 mutant flies lacking melatonin had no circadian rhythmicity and >20% elevation in carbonyls. The rosy mutant, ry^{506} lacks urate, is hypersensitive to oxidants, expresses elevated carbonyls and has a phase shift [327].

Strong linkage of the clock to aging is revealed by $Bmal1^{-/-}$ mice. In addition to disruption of circadian behavior and transcriptional rhythms these mice express accelerated aging and dramatically reduced growth. Symptoms include reduced lifespan (~1 year), sarcopenia and organ wasting, cataracts, reduced subcutaneous fat, slow hair growth and radiation sensitivity [328]. Free radical processes were elevated in heart, kidney and spleen in association with organ shrinkage. A linkage of *Bmal1* to insulin sensitivity suggests a connection to the PI3K/insulin-IGF-1 pathway known to importantly modulate aging rates. The authors argue that a strong role in free radical/redox regulation for the clock would be expected due to the normal circadian variation in animal activity and metabolic rates [328].

Although RONS can directly inhibit transcription of TH and DA synthesis, failure of electroplasmic cycling in such strongly circadian neurons could result in a temporal stall or distortion that blocks access to the transcriptional window. Distortions in redox-sensitive regulatory switches could occur throughout the cell. Such effects might arise from disruption of the master circadian clock, sleep-wake cycles that are closely associated with DA rhythms, or from intracellular alterations associated with aging and/or stress that disrupt onboard clock function. Moreover, potentially antagonistic aspects of DA regulatory circuitry (e.g., receptor expression, DA synthesis, transport, secretion, reuptake and degradation) are relegated to different temporal windows requiring sequential coordination for proper functioning.

Circadian rhythmicity has been documented in many aspects of DA-regulated motor functions (including PD symptoms) and emotional, cognitive and sleep impacts associated with dopaminergic drugs. Symptoms of PD are ameliorated by sleep [318, 329]. Mice lacking a functional *Clock* gene showed increased cocaine-induced reward and excitability of DA neurons in the VTA. This was associated with increased gene expression and phosphorylation of TH suggesting that TH is closely controlled by CLOCK [318].

Arousal in Drosophila is mediated by DA [319]. Behavioural sensitization of *Drosophila* to cocaine required expression of several clock genes. PER, in conjunction with CLOCK and CYCLE, regulated expression of tyrosine decarboxylase [330]. *Per 2* gene expression declined with age, but *Per 1* did not [see 331]. Although mice deficient in *Per 1* and *Cry 2* initially maintained a circadian rhythm, this was lost in 87% of year old mice. Mice show reduced amplitude in wheel running, fragmentation of behavior, decreased precision in daily activation and defective photic signalling to the SCN. Defects included blunted expression of arginine vasopressin, the key output signal of the clock. The authors interpreted this as a form of accelerated clock aging [331].

Aged mouse lemurs showed increased daytime activity and shorter free-running rhythms. Peaks in arginine vasopressin and vasoactive intestinal peptide were shifted. Changes in rhythms of these hormones could impair SCN signaling [332]. Spontaneous motor activity during the scotophase declined in aged rats and the activity pattern suggested loss of circadian amplitude. This was associated with declining DA synthesis (~30%) in the striatum and nucleus accumbens from 3 to 28 months of age, although DA concentrations were maintained [45]. Of potential importance, DA rhythmicity in the tuberoinfundibular DA system, which is less stressed than the nigrostriatal system, disappeared in aged rats [333]. Given that TH transcription is activity dependent, and may be reduced or lost in quiescence, loss could occur in arrhythmic neurons.

Sleep pathology in PD involves daytime hypoactivity and sleeping. Alternatively, the sleep-period is fragmented and insomnia may be associated with motor engagement. Such symptoms may be accompanied by depression [334] and may precede overt PD [335]. Suppression of motor activity (i.e., limb movements, sleep walking) involves pathways linking midbrain DA to pre-motor areas [299]. In primates treated with MPTP sleepiness is reversed by L-DOPA and DA reuptake blockade, but not by DA_{r2} agonists [336]. *Drosophila* express age-related sleep

fragmentation resembling aging mammals. This possibly reflects RONS damage to central regulatory systems [337].

In mammals TH is lowest several hours prior to waking and peaks in association with maximal DA and motor activity. Alterations in temporal dynamics could alter sleep and contribute to excessive movement in PD and restless legs syndrome. Linkage of DA to sleepiness likely involves inhibition of VTA neurons. DAT critically determines synaptic DA levels and is expressed temporally opposite to other DA system elements. Wake-promoting effects of drugs like amphetamine involve enhanced synaptic DA via regulation of DAT and DAT-deficient mice fail to show drug-mediated wakefulness [299]. DAT is usually considered in its role of terminating DA synaptic signalling via DA reuptake, but expression of DAT in sleep has the larger function of clearing wake-associated DA from the sleeping milieu.

TH gene expression was upregulated in the rat SN and VTA during the diurnal period (a few hours before the lights-off waking period). Note that this may coincide with low protein levels. In the caudate putamen DA_{r2} expression was reduced diurnally and elevated at night. Expression of TH at the end of the sleep cycle may ensure abundant DA during waking and may contribute to improved early waking functions in PD [179].

Individuals with sleep apnea may show continued daytime sleepiness despite amelioration of the condition. This traces to loss of wake-active neurons that are sensitive to repeated hypoxia-reoxygenation [338]. Long-term exposure of mice to hypoxia-reoxygenation impaired daytime waking. Neurons impacted included DA in the ventral periaqueductal gray and NE in the locus ceruleus. Other neurotransmitter systems were unaffected. Six months of exposure led to 40% loss of catecholaminergic wake neurons. Daytime sleepiness is a reliable biomarker of PD, and to a lesser extent, normal aging. Loss of DA and locus ceruleus neurons involved in arousal and locomotion is an early event in both PD and AD [338]. Loss of arousal and increased daytime sleepiness could also predispose to obesity which exacerbates sleep apnea.

NOX on catecholamine wake neurons was strongly associated with hypoxia-reoxygenation. Activated NOX subunits translocated to the mitochondria, ER and cell membrane. The NOX inhibitor apocynin was protective and mice lacking functional NOX were resistant [338]. NOX expression was weak in other neuronal types. DA neurons are highly sensitive to RONS which likely exacerbates loss in sleep apnea [339].

There is a perception that DA neurons exist in a constant state of oxidative stress associated with RONS production [e.g., 340]. In fact, they are inactive in sleep. Repeated waking associated with sleep apnea could be damaging but it is more likely that disruption of sleep-associated anabolic functions (e.g., synaptic and neuronal restoration, detoxification, regeneration of antioxidants, restoration of NAD(P)H, and reduction or replacement of proteins expressing redox-sensitive cysteine/methionine residues) is as detrimental to high-metabolism DA neurons as is activity itself.

Future Directions

Electroplasmic theory highlights temporal compartmentalization of functions and the need to maintain robust rhythms and balance. Thus, aging of the dopaminergic system may trace just as strongly to restorative phases of the cycle as to neuronal activity. Supplementing with one cocktail at night might promote detoxification and recharging, whereas another type of supplement might be warranted for active mitochondria. Given the core role of redox it is important to establish whether aging tissues are biased towards oxidative stress, hypoxia, or both. Or are they just going flatline? The critical involvement of ions means that they may be as valuable as antioxidants in managing aging. We need more specific drugs. The pervasive regulatory role of redox-sensitive cysteines needs to be fully explored. The potential for products of DA metabolism and peroxidation to bind these sites is profound. We need to examine global redox and signaling implications. How changing HPA/GH balance relates to this framework will add another important overlay. Finally, the electroplasmic status of stem cells is crucial since it is their activity that forestalls real aging.

References

1. Rollo CD (1994) Phenotypes: their epigenetics, ecology and evolution. Chapman and Hall, London, pp 1–463
2. Finch CE (1990) Longevity, senescence and the genome. University of Chicago Press, Chicago, pp 1–922
3. Knoll J (1988) The striatal dopamine dependency of life span in male rats. Longevity study with (−)deprenyl. Mech Ageing Dev 46:237–262
4. Cardozo-Pelaez F, Brooks PJ, Stedeford T et al (2000) DNA damage, repair, and antioxidant systems in brain regions: a correlative study. Free Radical Biol Med 28:779–785
5. Reeves S, Bench C, Howard R (2002) Ageing and the nigrostiatal dopaminergic system. Int J Geriatr Psychiatry 17: 359–370
6. Evans MD, Dizdaroglu M, Cooke MS (2004) Oxidative DNA damage and disease: induction, repair and significance. Mut Res 567:1–61
7. Thomas B, Beal MF (2007) Parkinson's disease. Human Mol. Genet 16:R183–R194
8. Halliwell B (2006) Proteasomal dysfunction: a common feature of neurodegenerative diseases? Implications for the environmental origins of neurodegeneration. Antioxid Redox Signal 8:2007–2019

9. Luo Y, Roth GS (2000) The roles of dopamine oxidative stress and dopamine receptor signaling in aging and age-related neurodegeneration. Antiox Redox Signal 2:449–460
10. Greene JG, Dingledine R, Greenamyre JT (2005) Gene expression profiling of rat midbrain dopamine neurons: implications for selective vulnerability in parkinsonism. Neurobiol Dis 18:19–31
11. Schipper HM, Liberman A, Stopa EG (1998) Neural heme oxygenase-1 expression in idiopathic Parkinson's disease. Exp Neurol 150:60–68
12. Gao HM, Liu B, Hong JS (2003) Critical role for microglial NADPH oxidase in rotenone-induced degeneration of dopaminergic neurons. J Neurosci 23:6181–6187
13. Borges CR, Geddes T, Watson JT et al (2002) Dopamine biosynthesis is regulated by S-glutathionylation: potential mechanism of tyrosine hydroxylase inhibition during oxidative stress. J Biol Chem 277:48295–48302
14. Neckameyer WS, Woodrome S, Holt B et al (2000) Dopamine and senescence in *Drosophila melanogaster*. Neurobiol Aging 21:145–152
15. De La Cruz CP, Revilla E, Venero JL et al (1996) Oxidative inactivation of tyrosine hydroxylase in substantia nigra of aged rat. Free Radical Biol Med 20:53–61
16. Goudsmit E, Feenstra MGP, Swaab DF (1990) Central monoamine metabolism in the male brown-Norway rat in relation to aging and testosterone. Brain Res Bull 25:755–763
17. Cruz-Muros I, Afonso-Oramas D, Abreu P et al (2007) Aging of the rat mesostriatal system: Differences between the nigrostriatal and the mesolimbic compartments. Exp Neurol 204:147–161
18. Morgan DG, May PC, Finch CE (1987) Dopamine and serotonin systems in human and rodent brain: effects of age and neurodegenerative disease. J Am Geriatr Soc 35:334–345
19. McGeer PL, McGeer EG, Suzuki JS (1977) Aging and extrapyramidal function. Arch Neurol 34:33–35
20. Fearnley JM, Lees AJ (1991) Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain 114:2283–2301
21. Ma SY, Roytt M, Collan Y et al (1999) Unbiased morphometrical measurements show loss of pigmented nigral neurones with ageing. Neuropathol Appl Neurobiol 25:394–399
22. Stark AK, Pakkenberg B (2004) Histological changes of the dopaminergic nigrostriatal system in aging. Cell Tissue Res 318:81–92
23. Backman L, Nyberg L, Lindenberger U et al (2006) The correlative triad among aging, dopamine, and cognition: Current status and future prospects. Neurosci Biobehav Rev 30:791–807
24. Beach TG, Sue LI, Walker DG et al (2007) Marked microglial reaction in normal aging human substantia nigra: correlation with extraneuronal neuromelanin pigment deposits. Acta Neuropathol 114:419–424
25. Backman L, Farbe L (2004) The role of dopamine systems in cognitive aging. In: Cabeza R, Nyberg L, Park D (eds) Cognitive neuroscience of aging: linking cognitive and cerebral aging. Oxford University Press, New York, pp 58–84
26. Wu DC, Teismann P, Tieu K et al (2003) NADPH oxidase mediates oxidative stress in the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine model of Parkinson's disease. PNAS 100:6145–6150
27. Ross GW, Petrovitch H, Abbott RD et al (2004) Parkinsonian signs and substantia nigra neuron density in decendents elders without PD. Ann Neurol 56:532–539
28. Rudow G, O'Brien R, Savonenko AV et al (2008) Morphometry of the human substantia nigra in ageing and Parkinson's disease. Acta Neuropathol (Online). doi:10.1007/s00401-008-0352-8
29. Cabello CR, Thune JJ, Pakkenberg H et al (2002) Ageing of substantia nigra in humans: cell loss may be compensated by hypertrophy. Neuropathol Appl Neurobiol 28:283–291
30. Kubis N, Faucheux BA, Ransmayr G et al (2000) Preservation of midbrain catecholaminergic neurons in very old human subjects. Brain 123:366–373
31. Wolf ME, LeWitt PA, Bannon MJ et al (1991) Effect of aging on tyrosine hydroxylase protein content and the relative number of dopamine nerve terminals in human caudate. J Neurochem 56:1191–1200
32. Eidelberg D, Takikawa S, Dhawan V et al (1993) Striatal 18F-dopa uptake: absence of an aging effect. J Cereb Blood Flow Metab 13:881–888
33. Rapp PR, Gallagher M (1996) Preserved neuron number in the hippocampus of aged rats with spatial learning deficits. PNAS 93:9926–9930
34. Rapp PR, Deroche PS, Mao Y et al (2002) Neuron number in the parahippocampal region is preserved in aged rats with spatial learning deficits. Cerebral Cortex 12:1171–1179
35. Keuker JIH, Luiten PGM, Fuchs E (2003) Preservation of hippocampal neuron numbers in aged rhesus monkeys. Neurobiol Aging 24:157–165
36. McCormack AL, Di Monte DA, Delfani K et al (2004) Aging of the nigrostriatal system in the squirrel monkey. J Comp Neurol 471:387–395
37. Dickstein DL, Kabaso D, Rocher AB et al (2007) Changes in the structural complexity of the aged brain. Aging Cell 6:275–284
38. Toescu EC, Verkhratsky A (2007) The importance of being subtle: small changes in calcium homeostasis control cognitive decline in normal aging. Aging Cell 6:267–273
39. Peters A, Rosene DL, Moss MB et al (1996) Neurobiological bases of age-related cognitive decline in the rhesus monkey. J Neuropathol Exp Neurol 55:861–874
40. Pakkenberg H, Anderson BB, Burns RS et al (1995) A stereological study of substantia nigra in young and old rhesus monkeys. Brain Res 693:201–206
41. Javoy-Agid F, Hirsch EC, Dumas S et al (1990) Decreased tyrosine hydroxylase messenger RNA in the surviving dopamine neurons of the substantia nigra in Parkinson's disease: an in situ hybridization study. Neuroscience 38:245–253
42. Kastner A, Hirsch EC, Agid Y et al (1993) Tyrosine hydroxylase protein and messenger RNA in the dopaminergic nigral neurons of patients with Parkinson's disease. Brain Res 606:341–345
43. Anglade P, Vyas S, Hirsch EC et al (1997) Apoptosis in dopaminergic neurons of the human substantia nigra during normal aging. Histol Histopathol 12:603–610
44. Tompkins MM, Basgall EJ, Zamrini E et al (1997) Apoptotic-like changes in Lewy-body-associated disorders and normal aging in substantia nigral neurons. Am J Pathol 150:119–131
45. Watanabe H (1987) Differential decrease in the rate of dopamine synthesis in several dopaminergic neurons of aged rat brain. Exp Gerontol 22:17–25
46. Kish SJ, Shannak K, Rajput A et al (1992) Aging produces a specific pattern of striatal dopamine loss: implications for the etiology of idiopathic Parkinson's disease. J Neurochem 58:642–648
47. Thannickal TC, Lai YY, Siegel JM (2008) Hypocretin (orexin) and melanin concentrating hormone loss and the symptoms of Parkinson's disease. Brain 131:e87 (Online). doi:10.1093/brain/awm221
48. Haycock JW, Becker L, Ang L et al (2003) Marked disparity between age-related changes in dopamine and other presynaptic dopaminergic markers in human striatum. J Neurochem 87:574–585

49. Gerhardt GA, Cass WA, Yi A et al (2002) Changes in somatodendritic but not terminal dopamine regulation in aged rhesus monkeys. J Neurochem 80:168–177
50. Bannon MJ, Poosch MS, Xia Y et al (1992) Dopamine transporter mRNA content in human substantia nigra decreases precipitously with age. PNAS 89:7095–7099
51. Kish SJ, Zhong XH, Hornykiewicz O et al (1995) Striatal 3,4-dihydroxyphenylalanine decarboxylase in aging: disparity between postmortem and positron emission tomography studies? Ann Neurol 38:260–264
52. Bohnen NI, Albin RL, Koeppe RA et al (2006) Positron emission tomography of monoaminergic vesicular binding in aging and Parkinson disease. J Cerebral Blood Flow Metab 26:1198–1212
53. Bannon MJ, Whitty CJ (1997) Age-related and regional differences in dopamine transporter mRNA expression in human midbrain. Neurology 48:969–977
54. Volkow ND, Gur RC, Wang GJ et al (1998) Association between decline in brain dopamine activity with age and cognitive and motor impairment in healthy individuals. Am J Psychiatry 155:344–349
55. van Dyck CH, Seibyl JP, Malison RT et al (1995) Age-related decline in striatal dopamine transporter binding with iodine-123-ß-CITSPECT. J Nuclear Med 36:1175–1181
56. van Dyck CH, Seibyl JP, Malison RT (2002) Age related decline in dopamine transporters: Analysis of striatal subregions, nonlinear effects, and hemispheric asymmetries. Am J Geriatr Psychiatry 10:36–43
57. Erixon-Lindroth N, Farde L, Wahlin TBR et al (2005) The role of the striatal dopamine transporter in cognitive aging. Psychiatry Res Neuroimag 138:1–12
58. Castner SA, Goldman-Rakic PS (2004) Enhancement of working memory in aged monkeys by a sensitizing regimen of dopamine D_1 receptor stimulation. J Neurosci 24:1446–1450
59. Volkow ND, Logan J, Fowler JS et al (2000) Association between age-related decline in brain dopamine activity and impairment in frontal and cingulate metabolism. Am J Psychiatry 157:75–80
60. Rollo CD (2007) Speculations on the evolutionary ecology of *Homo sapiens* with special reference to body size, allometry and survivorship. In: Samaras T (ed) Human body size and the laws of scaling. Nova Biomedical Publishers, New York, pp 261–299
61. Vallender EJ, Lahn BT (2004) Positive selection on the human genome. Hum Mol Genet 13:R245–R254
62. Preuss TM, Caceres M, Oldham MC et al (2004) Human brain evolution: insights from microarrays. Nat Rev Genet 5:850–860
63. Williams GC (1957) Pleiotropy, natural selection, and the evolution of senescence. Evolution 11:398–411
64. Rollo CD (2002) Growth negatively impacts the life span of mammals. Evol Dev 4:55–61
65. Rollo CD (2007) Overview of research on giant transgenic mice with emphasis on the brain and aging. In: Samaras T (ed) Human body size and the laws of scaling. Nova Biomedical Publishers, New York, pp 235–260
66. Vermeulen CJ, Loeschcke V (2007) Longevity and the stress response in *Drosophila*. Exp Gerontol 42:153–159
67. Allain H, Bentue-Ferrer D, Akwa Y (2008) Disease-modifying drugs and Parkinson's disease. Prog Neurobiol 84:25–39
68. Cotzias GC, Miller ST, Nicholson AR (1974) Prolongation of the life-span in mice adapted to large doses of L-DOPA. PNAS 71:2466–2469
69. Cotzias GC, Miller ST, Tang LC et al (1977) Levodopa, fertility, and longevity. Science 196:549–551
70. Chihara K, Kashio Y, Kita T et al (1986) L-DOPA stimulates release of hypothalamic growth hormone-releasing hormone in humans. J Clin Endocrinol Metab 62:466–473
71. De la Cruz CP, Revilla E, Rodríguez-Gomez JA et al (1997) (−)-Deprenyl treatment restores serum insulin-like growth factor-I (IGF-I) levels in aged rats to young rat level. Eur J Pharmacol 327:215–220
72. Herenu CB, Cristina C, Rimoldi OJ et al (2007) Restorative effect of insulin-like growth factor-I gene therapy in the hypothalamus of senile rats with dopaminergic dysfunction. Gene Therapy 14:237–245
73. Diaz-Torga G, Feierstein C, Libertun C et al (2002) Disruption of the D2 dopamine receptor alters GH and IGF-I secretion and causes dwarfism in male mice. Endocrinoloy 143:1270–1279
74. De Luca M, Rose G, Bonafe M et al (2001) Sex-specific longevity associations defined by tyrosine hydroxylase–insulin–insulin growth factor 2 haplotypes on the 11p15.5 chromosomal region. Exp Gerontol 36:1663–1671
75. Kitani K, Kanai S, Carrillo MC et al (1994) (−)Deprenyl increases the life span as well as activities of superoxide dismutase and catalase but not of glutathione peroxidase in selective brain regions in Fischer rats. Ann NY Acad Sci 717:60–71
76. Carter CS, Sonntag WE, Onder G et al (2002) Physical performance and longevity in aged rats. J Gerontol Ser A: Biol Sci Med Sci 57:B193–B197
77. Joseph JA, Bartus RT, Clody D et al (1983) Psychomotor performance in the senescent rodent: reduction of deficits via striatal dopamine receptor up-regulation. Neurobiol Aging 4:313–319
78. Vermeulen CJ, Cremers T, Westerink BHC et al (2006) Changes in dopamine levels and locomotor activity in response to selection on virgin lifespan in *Drosophila melanogaster*. Mech Ageing Dev 127:610–617
79. De Luca M, Roshina NV, Geiger-Thornsberry GL et al (2003) Dopa decarboxylase (*Ddc*) affects variation in *Drosophila* longevity. Nat Genet 34:429–433
80. Hendricks JC, Lu S, Kume K (2003) Gender dimorphism in the role of cycle (BMAL1) in rest, rest regulation, and longevity in *Drosophila melanogaster*. J Biol Rhythms 18:12–25
81. Bradley AJ, McDonald IR, Lee AK (1980) Stress and mortality in a small marsupial (*Antechinus stuartii*, Macleary). Gen Comp Endocrinol 40:188–200
82. Naylor E, Bergmann BM, Krauski K et al (2000) The circadian Clock mutation alters sleep Homeostasis in the Mouse. J Neurosci 20:8138–8143
83. Meites J (1995) Neuroendocrine control of reproduction in aging rats and humans. In: Sarkar DK, Barnes CD (eds) The reproductive endocrinology of aging and drug abuse. CRC Press, Boca Raton, pp 109–168
84. Forman LJ, Sonntag WE, Miki N et al (1980) Maintenance by L-DOPA treatment of estrous cycles and LH response to estrogen in aging female rats. Exp Aging Res 6:547–554
85. Yin W, Gore AC (2006) Neuroendocrine control of reproductive aging: roles of GnRH neurons. Reproduction 131:403–414
86. Simpkins JW, Mueller GP, Huang HH et al (1977) Evidence for depressed catecholamine and enhanced serotonin metabolism in aging male rats: possible relation to gondotropin secretion. Endocrinol 100:1672–1678
87. Prasad SK, Qureshi TN, Saxena S et al (2007) L-Dopa feeding induces body growth and reproductive conditions in Japanese quail, Coturnix coturnix Japonica. Int J Poultry Sci 6:560–566
88. Tsai HW, Shui HA, Liu HS et al (2006) Monoamine levels in the nucleus accumbens correlate with male sexual behavior in middle-aged rats. Pharmacol Biochem Behav 83:265–270
89. Hussain T, Lokhandwala MF (1998) Renal dopamine receptor function in hypertension. Hypertension 32:187–197
90. Zeng C, Yang Z, Wang Z et al (2005) Interaction of angiotensin II type 1 and D_5 dopamine receptors in renal proximal tubule cells. Hypertension 45:804–810

91. Banday AA, Lokhandwala MF (2007) Oxidative stress reduces renal dopamine D1 receptor-$G_{q/11}\alpha$ G protein-phospholipase C signaling involving G protein-coupled receptor kinase 2. Am J Physiol Renal Physiol 293:F306–F315
92. Steiner B, Winter C, Hosman K et al (2006) Enriched environment induces cellular plasticity in the adult substantia nigra and improves motor behavior function in the 6-OHDA rat model of Parkinson's disease. Exp Neurol 199:291–300
93. Lemon JA, Boreham DR, Rollo CD (2003) A dietary supplement abolishes age-related cognitive decline in transgenic mice expressing elevated free radical processes. Exp Biol Med 228:800–810
94. O'Rourke B, Cortassa S, Aon MA (2005) Mitochondrial ion channels: gatekeeprs of life and death. Physiology 20:303–315
95. Gibson GE, Peterson C (1987) Calcium and the aging nervous system. Neurobiol Aging 8:329–343
96. Mattson MP (2007) Calcium and neurodegeneration. Aging Cell 6:337–350
97. Thibault O, Gant JC, Landfield PW (2007) Expansion of the calcium hypothesis of brain aging and Alzheimer's disease: minding the store. Aging Cell 6:307–317
98. Squier TC (2001) Oxidative stress and protein aggregation during biological aging. Exp Gerontol 36:539–1550
99. Beal MF (1998) Excitotoxicity and nitric oxide in Parkinson's disease pathogenesis. Ann Neurol 44(Suppl 1):S110–S114
100. Tang TS, Slow E, Lupu V et al (2005) Disturbed Ca^{2+} signaling and apoptosis of medium spiny neurons in Huntington's disease. PNAS 102:2602–2607
101. Yan Y, Wei CL, Zhang WR et al (2006) Cross-talk between calcium and reactive oxygen species signaling. Acta Pharmacol Sinica 27:821–826
102. Kourie JI (1998) Interaction of reactive oxygen species with ion transport mechanisms. Am J Physiol Cell Physiol 275: C1–C24
103. Rollo CD (2007) Multidisciplinary aspects of regulatory systems relevant to multiple stressors: aging, xenobiotics and radiation. In: Mothersill C, Mosse I, Seymour C (eds) Multiple stressors: a challenge for the future. Springer, Dordrecht, pp 185–224
104. Koliwad SK, Elliott SJ, Kunze DL (1996) Oxidized glutathione mediates cation channel activation in calf vascular endothelial cells during oxidant stress. J Physiol 495:37–49
105. Wang JW, Humphreys JM, Phillips JP et al (2000) A novel leg-shaking *Drosophila* mutant defective in a voltage-gated K^+ current and hypersensitive to reactive oxygen species. J Neurosci 20:65958–65964
106. Matalon S, Hardiman KM, Jain L et al (2003) Regulation of ion channel structure and function by reactive oxygen–nitrogen species. Am J Physiol Lung Cell Mol Physiol 285:L1184–L1189
107. Avshalumov MV, Chen BT, Marshall SP et al (2003) Glutamate-dependent inhibition of dopamine release in striatum is mediated by a new diffusible messenger, H_2O_2. J Neurosci 23:2744–2750
108. Pletjushkina OY, Fetisova EK, Lyamzaev KG et al (2005) Long-distance apoptotic killing of cells is mediated by hydrogen peroxide in a mitochondrial ROS-dependent fashion. Cell Death Differ 12:1442–1444
109. Ichinari K, Kakei M, Matsuoka T et al (1996) Direct activation of the ATP-sensitive potassium channel by oxygen free radicals in guinea-pig ventricular cells: its potentiation by MgADP. J Mol Cell Cardiol 28:1867–1877
110. Beckman KB, Ames BN (1998) The free radical theory of aging matures. Physiol Rev 78:547–581
111. Liu J (2008) The effects and mechanisms of mitochondrial nutrient α-lipoic acid on improving age-associated mitochondrial and cognitive dysfunction: an overview. Neurochem Res 33:194–203
112. Lutz PL, Prentice HM, Milton SL (2003) Is turtle longevity linked to enhanced mechanisms for surviving brain anoxia and reoxygenation? Exp Gerontol 38:797–800
113. McCartney CE, McClafferty H, Huibant JM et al (2005) A cysteine-rich motif confers hypoxia sensitivity to mammalian large conductance voltage- and Ca-activated K (BK) channel α -subunits. PNAS 102:17870–17876
114. Brown MF, Gratton TP, Stuart JA (2007) Metabolic rate does not scale with body mass in cultured mammalian cells. Am J Physiol Regul Integr Comp Physiol 292:R2115–R2121
115. Disterhoft JF, Oh MM (2007) Alterations in intrinsic neuronal excitability during normal aging. Aging Cell 6:327–336
116. Thibault O, Landfield PW (1996) Increase in single L-type calcium channels in hippocampal neurons during aging. Science 272:1017–1020
117. Yamada T, McGeer PL, Baimbridge KG et al (1990) Relative sparing in Parkinson's disease of substantia nigra dopamine neurons containing calbindin-D_{28K}. Brain Res 526:303–307
118. Kamsler A, Segal M (2003) Hydrogen peroxide modulation of synaptic plasticity. J Neurosci 23:269–276
119. Davare MA, Hell JW (2003) Increased phosphorylation of the neuronal L-type Ca^{2+} channel $Ca_v1.2$ during aging. PNAS 100:16018–16023
120. Batuecas A, Pereira R, Centeno C et al (1998) Effects of chronic nimodipine on working memory of old rats in relation to defects in synaptosomal calcium homeostasis. Eur J Pharmacol 350:141–150
121. Norris CM, Halpain S, Foster TC (1998) Reversal of age-related alterations in synaptic plasticity by blockade of L-Type Ca^{2+} channels. J Neurosci 18:3171–3179
122. Tzounopoulos T, Stackman R (2003) Enhancing synaptic plasticity and memory: a role for small-conductance Ca^{2+}-activated K^+ channles. Neuroscientist 9:434–439
123. Gant JC, Sama MM, Landfield PW et al (2006) Early and simultaneous emergence of multiple hippocampal biomarkers of aging is mediated by Ca^{2+}-induced Ca^{2+} release. J Neurosci 26:3482–3490
124. Murphy GG, Fedorov NB, Giese KP et al (2004) Increased neuronal excitability, synaptic plasticity, and learning in aged $Kv\beta1.1$ knockout mice. Curr Biol 14:1907–1915
125. Horiuchi J, Saitoe M (2005) Can flies shed light on our own age-related memory impairment? Ageing Res Rev 4:83–101
126. Blalock EM, Chen KC, Sharrow K et al (2003) Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment. J Neurosci 23:3807–3819
127. Deyo RA, Straube KT, Disterhoft JF (1989) Nimodipine facilitates associative learning in aging rabbits. Science 243: 809–811
128. Markel E, Felszeghy K, Luiten PGM et al (1995) Beneficial effect of chronic nimodipine treatment on behavioral dysfunctions of aged rats exposed to perinatal ethanol treatment. Arch Gerontol Geriatr 21:75–88
129. Chapman PF (2005) Cognitive aging: recapturing the excitation of youth? Curr Biol 15:R31–R33
130. De Jong GI, Nyakas C, Scuurman T et al (1993) Aging-related alterations in behavioral activation and cerebrovascular integrity in rats are dose-dependently influenced by nimodipine. Neurosci Res Comm 12:1–8
131. Belforte JE, Magarinos-Azcone C, Armando I et al (2001) Pharmacological involvement of the calcium blocker flunarizine in dopamine transmission at the striatum. Parkinsonism Related Disord 8:33–40
132. Verkhratsky A (2005) Physiology and pathophysiology of the calcium store in the endoplasmic reticulum of neurons. Physiol Rev 85:201–279

133. Huddleston AT, Tang W, Takeshima H et al (2008) Superoxide-induced potentiation in the hippocampus requires activation of ryanodine receptor type 3 and ERK. J Neurophysiol 99:1565–1571
134. Hidalgo C, Carrasco MA, Munoz P et al (2007) A role for reactive oxygen/nitrogen species and iron on neuronal synaptic plasticity. Antioxid Redox Signal 9:245–255
135. Hammond RS, Bond CT, Strassmaier T et al (2006) Small-conductance Ca^{2+}-activated K^+ channel type 2 (SK2) modulates hippocampal learning, memory. and synaptic plasticity J Neurosci 26:1844–1853
136. Blank T, Nijholt I, Kye MJ et al (2003) Small-conductance, Ca^{2+}-activated K^+ channel SK3 generates age-related memory and LTP deficits. Nat Neurosci 6:911–912
137. Stackman RW, Hammond RS, Linardatos E et al (2002) Small conductance Ca^{2+}-activated K^+ channels modulate synaptic plasticity and memory encoding. J Neurosci 22:10163–10171
138. Brennan AR, Dolinsky B, Vu MAT et al (2008) Blockade of IP_3-mediated SK channel signaling in the rat medial prefrontal cortex improves spatial working memory. Learn Mem 15:93–96
139. Oh SW, Mukhopadhyay A, Dixit BL et al (2006) Identification of direct DAF-16 targets controlling longevity, metabolism and diapause by chromatin immunoprecipitation. Nat Genet 38:251–257
140. Lim D, Fedrizzi L, Tartari M et al (2008) Calcium homeostasis and mitochondrial dysfunction in striatal neurons of Huntington disease. J Biol Chem 283:5780–5789
141. Ghelardini C, Quattrone A, Galeotti N et al (2003) Antisense knockdown of the Shaker-like Kv1.1 gene abolishes the central stimulatory effects of amphetamines in mice and rats. Neuropsychopharmacology 28:1096–1105
142. Raimondi L, Alfarano C, Pacini A et al (2007) Methylamine-dependent release of nitric oxide and dopamine in the CNS modulates food intake in fasting rats. Brit J Pharmacol 150:1003–1010
143. Lawson K (2000) Is there a role for potassium channel openers in neuronal ion channel disorders? Expert Opin Invest Drugs 9:2269–2280
144. Vincent A, Buckley C, Schott JM et al (2004) Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. Brain 127:701–712
145. Michel PP, Alvarez-Fischer D, Guerreiro S et al (2007) Role of activity-dependent mechanisms in the control of dopaminergic neuron survival. J Neurochem 101:289–297
146. Liss B, Haeckel O, Wildmann J et al (2005) K-ATP channels promote the differential degeneration of dopaminergic midbrain neurons. Nat Neurosci 8:1742–1751
147. Liss B, Roeper J (2001) Molecular physiology of neuronal K-ATP channels (review). Mol Membr Biol 18:117–127
148. Avshalumov MV, Chen BT, Koos T et al (2005) Endogeneous hydrogen peroxide regulates the excitability of midbrain dopamine neurons via ATP-sensitive potassium channels. J Neurosci 25:4222–4231
149. Chen BT, Avshalumov MV, Rice ME (2001) H_2O_2 is a novel endogenous modulator of synaptic dopamine release. J Neurophysiol 85:2468–2476
150. Chen BT, Avshalumov MV, Rice ME (2002) Modulation of somatodendritic dopamine release by endogenous H_2O_2: susceptibility in substantia nigra but resistance in VTA. J Neurophysiol 87:1155–1158
151. Bao L, Avshalumov MV, Rice ME (2005) Partial mitochondrial inhibition causes striatal dopamine release suppression and medium spiny neuron depolarization via H_2O_2 elevation, not ATP depletion. J Neurosci 25:10029–10040
152. Rollo CD (2006) Radiation and the regulatory landscape of neo^2-Darwinism. Mut Res 597:18–31
153. Lamensdorf I, Meiri N, Harvey-White J et al (1999) Kir6.2 oligoantisense administered into the globus pallidus reduces apomorphine-induced turning in 6-OHDA hemiparkinsonian rats. Brain Res 818:275–284
154. Chan CS, Guzman JN, IIijic E et al (2007) 'Rejuvenation' protects neurons in mouse models of Parkinson's disease. Nature 447:1081–1086
155. Kuzhikandathil EV, Yu W, Oxford GS (1998) Human dopamine D3 and D2L receptors couple to inward rectifier potassium channels in mammalian cell lines. Mol Cellul Neurosci 12:390–402
156. Momiyama T, Koga E (2001) Dopamine D_2-like receptors selectively block N-type Ca^{2+} channels to reduce GABA release onto rat striatal cholinergic interneurons. J Physiol 533:479–492
157. Huang CL, Huang NK, Shyue SK et al (2003) Hydrogen peroxide induces loss of dopamine transporter activity: a calcium-dependent oxidative mechanism. J Neurochem 86:1247–1259
158. Sonders MS, Zhu SJ, Zahniser NR et al (1997) Multiple ionic conductances of the human dopamine transporter: the actions of dopamine and psychostimulants. J Neurosci 17:960–974
159. Berman SB, Zigmond MJ, Hastings TG (1996) Modification of dopamine transporter function: effect of reactive oxygen species and dopamine. J Neurochem 67:593–600
160. Morel P, Tallineau C, Pontcharraud R et al (1999) Effects of 4-hydroxynonenal, a lipid peroxidation product, on dopamine transport and Na^+/K^+ ATPase in rat striatal synaptosomes. Neurochem Intern 33:531–540
161. Salthun-Lassalle B, Hirsch EC, Wolfart J et al (2004) Rescue of mesencephalic dopaminergic neurons in culture by low-level stimulation of voltage-gated sodium channels. J Neurosci 24:5922–5930
162. Whitehead RE, Ferrer JV, Javitch JA et al (2001) Reaction of oxidized dopamine with endogenous cysteine residues in the human dopamine transporter. J Neurochem 76:1242–1251
163. Redman PT, Jefferson BS, Ziegler CB et al (2006) A vital role for voltage-dependent potassium channels in dopamine transporter-mediated 6-hydroxydopamine neurotoxicity. Neuroscience 143:1–6
164. Remillard CV, Yuan JXY (2004) Activation of K^+ channels: an essential pathway in programmed cell death. Am J Physiol Lung Cell Mol Physiol 286:L49–L67
165. Zhao YM, Sun LN, Zhou HY et al (2006) Voltage-dependent potassium channels are involved in glutamate-induced apoptosis of rat hippocampal neurons. Neurosci Lett 398:22–27
166. Bindoli A, Rigobello MP, Deeble DJ (1992) Biochemical and toxicological properties of the oxidation products of catecholamines. Free Radical Biol Med 13:391–405
167. Niznik HB, Fogel EF, Fassos FF et al (1991) The dopamine transporter is absent in Parkinsonian putamen and reduced in the caudate nucleus. J Neurochem 56:192–198
168. Call LM, Morton CM (2002) Continuing to break the sound barrier: genes in hearing. Cur Opin Genet Dev 12:343–348
169. Lee JW, Ryoo ZY, Lee EJ et al (2002) Circling mouse, a spontaneous mutant in the inner ear. Exp Anim 51:167–171
170. Wood JD, Muchinsky SJ, Filoteo AG et al (2004) Low endolymph calcium concentrations in $deafwaddler^{2J}$ mice suggest that PMCA2 contributes to endolymph calcium maintenance. J Assoc Res Otolaryngol 5:99–110
171. Cirelli C (2005) A molecular window on sleep: changes in gene expression between sleep and wakefulness. Neuroscientist 11:63–74
172. Kume K, Kume S, Park SK et al (2005) Dopamine is a regulator of arousal in the fruit fly. J Neurosci 25:7377–7384
173. Espinosa F, Marks G, Heintz N et al (2004) Increased motor drive and sleep loss in mice lacking Kv3-type potassium channels. Genes Brain Behav 3:90–100

174. Patil N, Cox DR, Bhat D et al (1995) A potassium channel mutation in weaver mice implicates membrane excitability in granuale cell differentiation. Nat Genet 11:126–129
175. Qiao X, Hefti F, Knusel B et al (1996) Selective failure of brain-derived neurotrophic factor mRNA expression in the cerebellum of *Stargazer*, a mutant mouse with ataxia. J Neurosci 16:640–648
176. Gutterman DD (2005) Mitochondria and reactive oxygen species: an evolution in function. Circul Res 97:302–304
177. Meng TC, Lou YW, Chen YY et al (2006) Cys-oxidation of protein tyrosine phosphatases: its role in regulation of signal transduction and its involvement in human cancers. J Cancer Mol 2:9–16
178. Tang XD, Daggett H, Hanner M et al (2001) Oxidative regulation of large conductance calcium-activated potassium channels. J Gen Physiol 117:253–274
179. Wei L, Yu SP, Gottron F et al (2003) Potassium channel blockers attenuate hypoxia- and ischemia-induced neuronal death in vitro and in vivo. Stroke 34:1281–1286
180. Katsuki H, Shibata H, Takenaka C et al (2003) *N*-methyl-D-aspartate receptors contribute to the maintenance of dopaminergic neurons in rat midbrain slice cultures. Neurosci Lett 341:123–126
181. Salthun-Lassalle B, Traver S, Hirsch EC et al (2005) Substance P, neurokinins A and B, and synthetic tachykinin peptides protect mesencephalioc dopaminergic neurons in culture via an activity-dependent mechanism. Mol Pharmacol 68:1214–1224
182. Yang F, Feng L, Zheng F et al (2001) GDNF acutely modulates excitability and A-type K^+ channels in midbrain dopaminergic neurons. Nat Neurosci 4:1071–1078
183. Douhou A, Troadec JD, Ruberg M et al (2001) Survival promotion of mesencephalic dopaminergic neurons by depolarizing concentrations of K^+ requires concurrent inactivation of NMDA or AMPA/kainate receptors. J Neurochem 78:163–174
184. Rossi D, Oshima T, Attwell D (2000) Glutamate release in severe brain ischaemia is mainly by reversed uptake. Nature 403:316–321
185. Shahar T, House SB, Gainer H (2004) Neural activity protects hypothalamic magnocellular neurons against axotomy-induced programmed cell death. J Neurosci 24:6553–6562
186. Grishin A, Ford H, Wang J et al (2005) Attenuation of apoptosis in enterocytes by blockade of potassium channels. Am J Physiol Gastrointest Liver Physiol 289:G815–G821
187. Andrews ZB, Horvath B, Barnstable CJ et al (2005) Uncoupling protein-2 is critical for nigral dopamine cell survival in a mouse model of Parkinson's disease. J Neurosci 25:184–191
188. Yu SP, Yeh CH, Strasser U et al (1999) NMDA Receptor-mediated K^+ efflux and neuronal apoptosis. Science 284:336–339
189. Adachi A, Nogi T, Ebihara S (1998) Phase-relationship and mutual effects between circadian rhythms of ocular melatoni and dopamine in the pigeon. Brain Res 792:361–369
190. McLaughlin BA, Pal S, Tran MP et al (2001) p38 activation is required upstream of potassium current enhancement and caspase cleavage in thiol oxidant-induced neuronal apoptosis. J Neurosci 21:3303–3311
191. Redman PT, He K, Hartnett KA et al (2007) Apoptotic surge of potassium currents is mediated by p38 phosphorylation of Kv2.1. PNAS 104:3568–3573
192. Pal S, Hartnett KA, Nerbonne JM et al (2003) Mediation of neuronal apoptosis by Kv2.1-encoded potassium channels. J Neurosci 23:4798–4802
193. Bossy-Wetzel E, Talantova MV, Lee W et al (2004) Crosstalk between nitric oxide and zinc pathways to neuronal cell death involving mitochondrial dysfunction and p38-activated K^+ channels. Neuron 41:351–365
194. Aizenman E, Stout AK, Hartnett KA et al (2000) Induction of neuronal apoptosis by thiol oxidation: putative role of intracellular zinc release. J Neurochem 75:1878–1888
195. Martin-Romero FJ, Santiago-Josefat B, Correa-Bordes J et al (2000) Potassium-induced apoptosis in rat cerebellar granule cells involves cell-cycle blockade at the G1/S transition. J Mol Neurosci 15:155–165
196. Verdaguer E, Jorda EG, Canudas AM et al (2003) 3-Amino thioacridone, a selective cyclin-dependent kinase 4 inhibitor, attenuates kainic acid-induced apoptosis in neurons. Neuroscience 120:599–603
197. Otsuka Y, Tanaka T, Uchida D et al (2004) Roles of cyclin-dependent kinase 4 and p53 in neuronal cell death induced by doxorubicin on cerebellar granule neurons in mouse. Neurosci Lett 365:180–185
198. Naetzker S, Hagen N, van Echten-Deckert G (2006) Activation of p38 mitogen-activated protein kinase and partial reactivation of the cell cycle by cis-4-methylsphingosine direct postmitotic neurons towards apoptosis. Genes Cells 11:269–279
199. Yu SP, Yeh CH, Sensi SL et al (1997) Mediation of neuronal apoptosis by enhancement of outward potassium current. Science 278:114–117
200. Platoshyn O, Zhang S, McDaniel SS et al (2002) Cytochrome *c* activates K^+ channels before inducing apoptosis. Am J Physiol Cell Physiol 283:C1298–C1305
201. Ekhterae D, Platoshyn O, Krick S et al (2001) Bcl-2 decreases voltage-gated K^+ channel activity and enhances survival in vascular smooth muscle cells. Am J Physiol Cell Physiol 281:C157–C165
202. Franklin JL, Sanz-Rodriguez C, Juhasz A et al (1995) Chronic depolarization prevents programmed death of sympathetic neurons in vitro but does not support growth: requirement for Ca^{2+} influx but not Trk activation. J Neurosci 15:643–664
203. Klatt P, Lamas S (2000) Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress. Eur J Biochem 267:4928–4944
204. Bigelow DJ, Squier TC (2005) Redox modulation of cellular signaling and metabolism through reversible oxidation of methionine sensors in calcium regulatory proteins. Biochim Biophys Acta Prot Proteom 1703:121–134
205. Stutzmann GE (2007) The pathogenesis of Alzheimers disease—is it a lifelong "Calciumopathy"? Neuroscientist 13:546–559
206. Ceriani MF, Hogenesch JB, Yanovsky M et al (2002) Genome-wide expression analysis in *Drosophila* reveals genes controlling circadian behavior. J Neurosci 22:9305–9319
207. Harman D (1996) Aging and disease: extending functional life span. Ann NY Acad Sci 786:321–336
208. Brand MD, Affourtit C, Esteves TC et al (2004) Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. Free Radical Biol Med 37:755–767
209. Keller JN, Gee J, Ding Q (2002) The proteasome in brain aging. Aging Res Rev 1:279–293
210. Keating DJ (2008) Mitochondrial dysfunction, oxidative stress, regulation of exocytosis and their relevance to neurodegenerative diseases. J Neurochem 104:298–305
211. Avshalumov MV, Rice ME (2002) NMDA receptor activation mediates hydrogen peroxide-induced pathophysiology in rat hippocampal slices. J Neurophysiol 87:2896–2903
212. Finkel T, Holbrook NJ (2000) Oxidants, oxidative stress and the biology of aging. Nature 408:239–247
213. Liu H, Colavitti R, Rovira II et al (2005) Redox-dependent transcriptional regulation. Circ Res 97:967–974
214. Droge W (2002) The plasma redox state and ageing. Ageing Res Rev 1:257–278
215. Droge W (2005) Oxidative aging and insulin receptor signaling. J Gerontol Biol Sci Med Sci 60(A):1378–1385

216. Klann E, Roberson ED, Knapp LT et al (1998) A role for superoxide in protein kinase C activation and induction of long-term potentiation. J Biol Chem 273:4516–4522
217. Hu D, Serrano F, Oury TD et al (2006) Aging-dependent alterations in synaptic plasticity and memory in mice that overexpress extracellular superoxide dismutase. J Neurosci 26:3933–3941
218. Finkel T (2003) Oxidant signals and oxidative stress. Curr Opin Cell Biol 15:247–254
219. Menon SG, Goswami PC (2007) A redox cycle within the cell cycle: ring in the old with the new. Oncogene 26:1101–1109
220. Rutter J, Reick M, Wu LC et al (2001) Regulation of Clock and NPAS2 DNA binding by the redox state of NAD cofactors. Science 293:510–514
221. Droge W, Schipper HM (2007) Oxidative stress and aberrant signaling in aging and cognitive decline. Aging Cell 6:361–370
222. Lloyd D, Murray DB (2007) Redox rhythmicity: clocks at the core of temporal coherence. Bioessays 29:465–473
223. Duffield GE, Best JD, Meurers BH et al (2002) Circadian programs of transcriptional activation, signaling and protein turnover revealed by microarray analysis of mammalian cells. Curr Biol 12:551–557
224. Serrano F, Klann E (2004) Reactive oxygen species and synaptic plasticity in the aging hippocampus. Ageing Res Rev 3:431–443
225. Ghezzi P, Bonetto V, Fratelli M (2005) Thiol–disulfide balance: from the concept of oxidative stress to that of redox regulation. Antioxid Redox Signal 7:964–972
226. Kishida KT, Klann E (2007) Sources and targets of reactive oxygen species in synaptic plasticity and memory. Antioxid Redox Signal 9:233–244
227. Lachmansingh E, Rollo CD (1994) Evidence for a trade off between growth and behavioural activity in giant "Supermice" genetically engineered with extra growth hormone genes. Can J Zool 72:2158–2168
228. Rollo CD, Foss J, Lachmansingh E et al (1997) Behavioral rhythmicity in transgenic growth hormone mice: trade-offs, energetics, and sleep-wake cycles. Can J Zool 75:1020–1034
229. Hajdu I, Obal F Jr, Fang J et al (2002) Sleep of transgenic mice producing excess rat growth hormone. Am J Physiol Regul Integr Comp Physiol 282:R70–R76
230. Tu BP, Kudlicki A, Rowicka M et al (2005) Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes. Science 310:1152–1158
231. Tu BP, Mohler RE, Liu JC et al (2007) Cyclic changes in metabolic state during the life of a yeast cell. PNAS 104:16886–16891
232. Jin X, Shearman LP, Weaver DR et al (1999) A molecular mechanism regulating rhythmic output from the suprachiasmatic circadian clock. Cell 96:57–68
233. Buijs RM, van Eden CC, Goncharuk VD et al (2003) The biological clock tunes the organs of the body: timing by hormones and the autonomic nervous system. J Endocrinol 177:17–26
234. Lowrey PL, Takahashi JS (2004) Mammalian circadian biology: elucidating genome-wide levels of temporal organization. Ann Rev Gen Hum Genet 5:407–441
235. Dioum EM, Rutter J, Tuckerman JR et al (2002) NPAS2: a gas-responsive transcription factor. Science 298:2385–2387
236. Doi M, Hirayama J, Sassone-Corsi P (2006) Circadian regulator CLOCK is a histone acetyltransferase. Cell 125:497–508
237. Claridge-Chang A, Wijinen H, Naef F et al (2001) Circadian regulation of gene expression systems in the *Drosophila* head. Neuron 32:657–671
238. Pando MP, Pinchak AB, Cermakian N et al (2001) A cell-based system that recapitulates the dynamic light-dependent regulation of the vertebrate clock. PNAS 98:10178–10183
239. Hirayama J, Cho S, Sassone-Corsi P (2007) Circadian control by the reduction/oxidation pathway: Catalase represses light-dependent clock gene expression in the zebrafish. PNAS 104:15747–15752
240. La Fleur SE, Kalsbeek A, Wortel J et al (2001) A daily rhythm in glucose tolerance: a role for the suprachiasmatic nucleus. Diabetes 50:1237–1243
241. Yin L, Wu N, Curtin JC et al (2007) Rev-erbα, a heme sensor that coordinates metabolic and circadian pathways. Science 318:1786–1789
242. Ryter SW, Tyrrell RM (2000) The heme synthesis and degradation pathways: role in oxidant sensitivity. Heme oxygenase has both pro- and antioxidant properties. Free Radical Biol Med 28:289–309
243. Etchegaray JP, Lee C, Wade PA et al (2003) Rhythmic histone acetylation underlies transcription in the mammalian circadian clock. Nature 421:177–182
244. Woelfle MA, Johnson CH (2006) No promoter left behind: global circadian gene expression in cyanobacteria. J Biol Rhythms 21:419–431
245. Shckorbatov YG, Zhuravlyova LA, Navrotskaya VV et al (2005) Chromatin structure and the state of human organism. Cell Biol Internat 29:77–81
246. Kondratov RV (2007) A role of the circadian system and circadian proteins in aging. Ageing Res Rev 6:12–27
247. Bordone L, Guarente L (2005) Calorie restriction, SIRT1 and metabolism: understanding longevity. Nat Rev Mol Cell Biol 6:298–305
248. Kang HL, Benzer S, Min KT (2002) Life extension in Drosophila by feeding a drug. PNAS 99:838–843
249. Noh KM, Koh JY (2000) Induction and activation by zinc of NADPH oxidase in cultured cortical neurons and astrocytes. J Neurosci 20:1–5
250. Tammariello SP, Quinn MT, Estus S (2000) NADPH oxidase contributes directly to oxidative stress and apoptosis in nerve growth factor-deprived sympathetic neurons. J Neurosci 20(RC53):1–5
251. Cai H (2005) NAD(P)H oxidase-dependent self-propagation of hydrogen peroxide and vascular disease. Circ Res 96:818–822
252. Lopez-Real A, Rey P, Soto-Otero R et al (2005) Angiotensin-converting enzyme inhibition reduces oxidative stress and protects dopaminergic neurons in a 6-hydroxydopamine rat model of parkinsonism. J Neurosci Res 81:865–873
253. Przedborski S, Ischiropoulos H (2005) Reactive oxygen and nitrogen species: weapons of neuronal destruction in models of Parkinson's disease. Antioxid Redox Signal 7:685–693
254. Rey P, Lopez-Real A, Sanchez-Iglesias S et al (2007) Angiotensin type-1-receptor antagonists reduce 6-hydroxydopamine toxicity for dopaminergic neurons. Neurobiol Aging 28:555–567
255. Morre DM, Lenaz G, Morre DJ (2000) Surface oxidase and oxidative stress propagation in aging. J Exp Biol 203:1513–1521
256. Morre DJ, Morre DM (2003) Cell surface NADH oxidases (ECTO-NOX proteins) with roles in cancer, cellular time keeping, growth, aging and neurodegenerative diseases. Free Radical Res 37:795–808
257. Orczyk J, Morre DM, Morre DJ (2005) Periodic fluctuations in oxygen consumption comparing HeLa (cancer) and CHO (non-cancer) cells and response to external $NAD(P)^+$/NAD(P)H. Mol Cell Biochem 273:161–167
258. Crane FL, Low H (2005) Plasma membrane redox and control of sirtuin. AGE 27:147–152
259. Kirsch M, De Groot H (2001) NAD(P)H, a directly operating antioxidant? FASEB J 15:1569–1574
260. Gery S, Komatsu N, Baldjyan L et al (2006) The circadian gene Per1 plays an important role in cell growth and DNA damage control in human cancer cells. Mol Cell 22:375–382

261. Burdon RH (1995) Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. Free Radical Biol Med 18:775–794
262. Ammendola R, Ruocchio MR, Chirico G et al (2002) Inhibition of NADH/NADPH oxidase affects signal transduction by growth factor receptors in normal fibroblasts. Arch Biochem Biophys 397:253–257
263. Miller BH, McDearmon EL, Panda S et al (2007) Circadian and CLOCK-controlled regulation of the mouse transcriptome and cell proliferation. PNAS 104:3342–3347
264. Froy O, Chapnik N, Miskin R (2006) Long-lived αMUPA transgenic mice exhibit pronounced circadian rhythms. Am J Physiol Endocrinol Metab 291:E1017–E1024
265. Kaasik K, Lee CC (2004) Reciprocal regulation of haem biosynthesis and the circadian clock in mammals. Nature 430:467–471
266. Venkatachalam P, de Toledo SM, Pandey BN et al (2008) Regulation of normal cell cycle progression by flavin-containing oxidases. Oncogene 27:20–31
267. Lambeth JD (2007) Nox enzymes, ROS, and chronic disease: an example of antagonistic pleiotropy. Free Radical Biol Med 43:332–347
268. Reddy AB, Karp NA, Maywood ES et al (2006) Circadian orchestration of the hepatic proteome. Curr Biol 16:1107–1115
269. Akhtar RA, Reddy AB, Maywood ES et al (2002) Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. Current Biol 12:540–550
270. Young MW (2004) An ultradian clock shapes genome expression in yeast. PNAS 101:1118–1119
271. Schibler U, Naef F (2005) Cellular oscillators: rhythmic gene expression and metabolism. Curr Opin Cell Biol 17:223–229
272. Sato TK, Panda S, Kay SA et al (2003) DNA arrays: applications and implications for circadian biology. J Biol Rhythms 18:96–105
273. Hunt T, Sassone-Corsi P (2007) Riding tandem: Circadian clocks and the cell cycle. Cell 129:461–464
274. Klevecz RR, Bolen J, Forrest G, Murray DB (2004) A genomewide oscillation in transcription gates DNA replication and cell cycle. PNAS 101:1200–1205
275. Chen Z, Odstrcil EA, Tu BP et al (2007) Restriction of DNA replication to the reductive phase of the metabolic cycle protects genome integrity. Science 316:1916–1919
276. De Monte S, d' Ovidio F, Dano S et al (2007) Dynamical quorum sensing: Population density encoded in cellular dynamics. PNAS 104:18377–18381
277. Desai VG, Moland CL, Branham WS et al (2004) Changes in expression level of genes as a function of time of day in the liver of rats. Mut Res 549:115–129
278. Brauer MJ, Huttenhower C, Airoldi EM et al (2008) Coordination of growth rate, cell cycle, stress response, and metabolic activity in yeast. Mol Biol Cell 19:352–367
279. Murray DB, Roller S, Kuriyama H et al (2001) Clock control of ultradian respiratory oscillation found during yeast continuous culture. J Bacteriol 183:7253–7259
280. Everson CA, Laatsch CD, Hogg N (2005) Antioxidant defense responses to sleep loss and sleep recovery. Am J Physiol Regul Integr Comp Physiol 288:R374–R383
281. Lloyd D, Eshantha L, Salgado J et al (2002) Respiratory oscillations in yeast: clock-driven mitochondrial cycles of energization. FEBS Lett 519:41–44
282. Lloyd D, Eshantha L, Salgado J et al (2002) Cycles of mitochondrial energization driven by the ultradian clock in a continuous culture of *Saccharomyces cerevisiae*. Microbiology 148:3715–3724
283. Murray DB, Engelen F, Lloyd D et al (1999) Involvement of glutathione in the regulation of respiratory oscillation during a continuous culture of *Saccharomyces cerevisiae*. Microbiology 145:2739–2745
284. Honda K, Komoda Y, Inoue S (1994) Oxidized glutathione regulates physiological sleep in unrestrained rats. Brain Res 14:253–258
285. Murray DB, Beckmann M, Kitano H (2007) Regulation of yeast oscillatory dynamics. PNAS 104:2241–2246
286. Salgado E, Murray DB, Lloyd D (2002) Some antidepressant agents (Li^+, monoamine oxidase type A inhibitors) perturb the ultradian clock in *Saccharomyces cerevisiae*. Biol Rhythm Res 33:351–361
287. Fu L, Pelicano H, Liu J, Huang P, Lee CC (2002) The circadian gene *Period2* plays an important role in tumor suppression and DNA damage response in vivo. Cell 111:41–50
288. McDonald MJ, Rosbash M (2001) Microarray analysis and organization of circadian gene expression in *Drosophila*. Cell 107:567–578
289. Maret S, Dorsaz S, Gurcel L et al (2007) *Homer1a* is a core brain molecular correlate of sleep loss. PNAS 104:20090–20095
290. Mackiewicz M, Shockley KR, Romer MA et al (2007) Macromolecule biosynthesis: a key function of sleep. Physiol Genomics 31:441–457
291. Jones S, Pfister-Genskow M, Benca RM et al (2008) Molecular correlates of sleep and wakefulness in the brain of the white-crowned sparrow. J Neurochem 105:46–62
292. Wright K (2002) Times of our lives. Sci Am 287:59–65
293. Mignot E, Taheri S, Nishino S (2002) Sleeping with the hypothalamus: emerging therapeutic targets for sleep disorders. Nat Neurosci 5:1071–1075
294. Cirelli C, Faraguna U, Tononi G (2006) Changes in gene expression after long-term sleep deprivation. J Neurochem 98:1632–1645
295. Fadel J, Deutch AY (2002) Anatomical substrates of orexin-dopamine interactions: lateral hypothalamic projections to the ventral tegmental area. Neuroscience 111:379–387
296. Korotkova TM, Sergeeva OA, Eriksson KS et al (2003) Excitation of ventral tegmental area dopaminergic and nondopaminergic neurons by orexins/hypocretins. J Neurosci 23:7–11
297. Alberto CO, Trask RB, Quinlan ME et al (2006) Bidirectional dopaminergic modulation of excitatory synaptic transmission in orexin neurons. J Neurosci 26:10043–10050
298. Narita M, Nagumo Y, Hashimoto S et al (2006) Direct involvement of orexinergic systems in the activation of the mesolimbic dopamine pathway and related behaviors induced by morphine. J Neurosci 26:398–405
299. Rye DB (2004) The two faces of Eve: Dopamine's modulation of wakefulness and sleep. Neurology 63:S2–S7
300. Weaver DR, Rivkees SA, Reppert SM (1992) D1-dopamine receptors activate c-fos expression in the fetal suprachiasmatic nuclei. PNAS 89:9201–9204
301. Michel S, Geusz ME, Zaritsky JJ et al (1993) Circadian rhythm in membrane conductance expressed in isolated neurons. Science 259:239–241
302. Manglapus MK, Iuvone PM, Underwood H et al (1999) Dopamine mediates circadian rhythms of rod-cone dominance in the Japanese quail retina. J Neurosci 19:4132–4141
303. Steenhard BM, Besharse JC (2000) Phase shifting the retinal circadian clock: xPer2 mRNA induction by light and dopamine. J Neurosci 20:8572–8577
304. Doyle SE, Grace MS, McIvor W et al (2002) Circadian rhythms of dopamine in mouse retina: the role of melatonin. Visual Neurosci 19:593–601

305. Green CB, Besharse JC (2004) Retinal circadian clocks and control of retinal physiology. J Biol Rhythms 19:91–102
306. Ribelayga C, Wang Y, Mangel SC (2003) A circadian clock in the fish retina regulates dopamine release via activation of melatonin receptors. J Physiol 554(2):467–482
307. Bartell PA, Miranda-Anaya M, McIvor W et al (2007) Interactions between dopamine and melatonin organize circadian rhythmicity in the retina of the green iguana. J Biol Rhythms 22:515–523
308. Tosini G, Davidson AJ, Fukuhara C et al (2007) Localization of a circadian clock in mammalian photoreceptors. FASEB J 21:3866–3871
309. Yujnovsky I, Hirayama J, Doi M et al (2006) Signaling mediated by the dopamine D2 receptor potentiates circadian regulation by CLOCK:BMAL1. PNAS 103:6386–6391
310. Yan L, Bobula JM, Svenningsson P et al (2006) DARPP-32 involvement in the photic pathway of the circadian system. J Neurosci 26:9434–9438
311. Djamgoz MBA, Hankins MW, Hirano J et al (1997) Neurobiology of retinal dopamine in relation to degenerative states of the tissue. Vision Res 37:3509–3529
312. Buttner T, Kuhn W, Patzold T, Przuntek H (1994) L-DOPA improves colour vision in Parkinson's disease. J Neural Transm 7:13–19
313. Muller T, Woitalla D, Peters S et al (2002) Progress of visual dysfunction in Parkinson's disease. Acta Neurol Scand 105:256–260
314. Lee JY, Djamgoz MBA (1997) Retinal dopamine depletion in young quail mimics some of the effects of ageing on visual function. Vision Res 37:1103–1113
315. Hankins MW (2000) Functional dopamine deficits in the senile rat retina. Visual Neurosci 17:839–845
316. Kunert KS, Fitzgerald ME, Thomson L et al (1999) Microglia increase as photoreceptors decrease in the aging avian retina. Curr Eye Res 18:440–447
317. Castaneda TR, de Prado BM, Prieto D et al (2004) Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: modulation by light. J Pineal Res 36:177–185
318. McClung CA, Sidiropoulou K, Vitaterna M et al (2005) Regulation of dopaminergic transmission and cocaine reward by the *Clock* gene. PNAS 28:9377–9381
319. Andretic R, van Swinderen B, Greenspan RJ (2005) Dopaminergic modulation of arousal in *Drosophila*. Curr Biol 15:1165–1175
320. Andretic R, Hirsh J (2000) Circadian modulation of dopamine receptor responsiveness in *Drosophila melanogaster*. PNAS 97:1873–1878
321. Feany MB, Bender WW (2000) A *Drosophila* model of Parkinson's disease. Nature 404:394–398
322. Hurd MW, Ralph MR (1998) The significance of circadian organization for longevity in the golden hamster. J Biol Rhythms 13:430–436
323. Oklejewicz M, Daan S (2002) Enhanced longevity in *Tau* mutant Syrian hamsters, *Mesocricetus auratus*. J Biol Rhythms 17:210–216
324. Cayetanot F, Perret M, Aujard F (2005) Shortened seasonal photoperiodic cycles accelerate aging of the diurnal and circadian locomotor activity rhythms in a primate. J Biol Rhythms 20:461–469
325. Hofman MA, Swaab DF (2006) Living by the clock: The circadian pacemaker in older people. Ageing Res Rev 5:33–51
326. Davidson AJ, Sellix MT, Daniel J et al (2006) Chronic jet-lag increases mortality in aged mice. Curr Biol 16:R914–R916
327. Coto-Montes A, Hardeland R (1999) Diurnal rhythm of protein carbonyl as an indicator of oxidative damage in *Drosophila melanogaster*: influence of clock gene alleles and deficiencies in the formation of free-radical scavengers. Biol Rhythm Res 30:383–391
328. Kondratov RV, Kondratova AA, Gorbacheva VY et al (2006) Early aging and age-related pathologies in mice deficient in BMAL1, the core componentof the circadian clock. Genes Dev 20:1868–1873
329. Weber M, Lauterburg T, Tobler I et al (2004) Circadian patterns of neurotransmitter related gene expression in motor regions of the rat brain. Neurosci Lett 358:17–20
330. Andretic R, Chaney S, Hirsh J (1999) Requirement of circadian genes for cocaine sensitization in *Drosophila*. Science 285:1066–1068
331. Oster H, Baeriswyl S, van der Horst GTJ et al (2003) Loss of circadian rhythmicity in aging mPer1$^{-/-}$ mCry2$^{-/-}$ mutant mice. Genes Dev 17:1366–1379
332. Aujard F, Cayetanot F, Bentivoglio M et al (2006) Age-related effects on the biological clock and its behavioral output in a primate. Chronobiol Int 23:451–460
333. Esquifino AI, Cano P, Jimenez V et al (2004) Changes of prolactin regulatory mechanisms in aging: 24-h rhythms of serum prolactin and median eminence and adenohypophysial concentration of dopamine, serotonin (γ-aminobutyric acid, taurine and somatostatin in young and aged rats. Exp Gerontol 39:45–52
334. Gjerstad MD, Wentzel-Larsen T, Aarsland D et al (2007) Insomnia in Parkinson's disease: frequency and progression over time. J Neurol Neurosurg Psychiatry 78:476–479
335. Thannickal TC, Lai YY, Siegel JM (2007) Hypocretin (orexin) cell loss in Parkinson's disease. Brain 130:1586–1595
336. Rye DB, Jankovic J (2002) Emerging views of dopamine in modulating sleep/wake state from an unlikely source: PD. Neurol 58:341–346
337. Koh K, Evans JM, Hendricks JC et al (2006) A *Drosophila* model for age-associated changes in sleep:wake cycles. PNAS 103:13843–13847
338. Zhu Y, Fenik P, Zhan G et al (2007) Selective loss of catecholaminergic wake-active neurons in a murine sleep apnea model. J Neurosci 27:10060–10071
339. Prevarskaya NB, Skryma RN, Vacher P et al (1995) Role of tyrosine phosphorylation in potassium channel activation. J Biol Chem 270:24292–24299
340. Sherer TB, Betarbet R, Stout AK et al (2002) An in vitro model of Parkinson's disease: linking mitochondrial impairment to altered alpha-synuclein metabolism and oxidative damage. J Neurosci 22:7006–7015