CHAPTER FIVE

# The Contribution of Mitochondria to Sensory Processing and Pain

**Sarah J.L. Flatters**[1]
Wolfson Centre for Age-Related Diseases, King's College London, London, United Kingdom
[1]Corresponding author: e-mail address: sarah.flatters@kcl.ac.uk

## Contents

1. Introduction 120
2. Role of Mitochondria in Normal Sensory Processing and Acute Pain 121
3. Clinical Evidence of Mitochondrial Dysfunction in Painful Conditions 126
4. Preclinical Evidence of Mitochondrial Dysfunction in Chronic Pain States 129
   - 4.1 Chemotherapy-induced painful neuropathy 129
   - 4.2 Diabetic painful neuropathy 134
   - 4.3 HIV-associated painful neuropathy 138
   - 4.4 Other chronic pain states 139
5. Future Perspectives 140

Acknowledgments 141
References 141

## Abstract

Mitochondria have a variety of essential functions within neurons including oxygen consumption, ATP generation, calcium buffering, and reactive oxygen species (ROS) generation. Despite extensive research into the contribution of mitochondrial function in other neurological disorders such as Parkinson's disease, the role of mitochondrial function in sensory processing and pain has been relatively unexplored until recent years. As this area of pain research is in its infancy, this review will be a descriptive summary—rather than a critical review—of data that suggests mitochondrial function/dysfunction as a causal or contributory mechanism of normal sensory processing and chronic pain. Evidence for mitochondrial dysfunction from both chronic pain patients and animal models of chronic pain will be described. Such evidence involves different aspects of mitochondria and their function including mitochondrial ultrastructure, distribution, oxygen consumption, oxidative phosphorylation, calcium buffering, ROS, and ATP levels. Most recently, substantial amounts of data have demonstrated mitochondrial involvement in painful peripheral neuropathies evoked by chemotherapy, diabetes, and HIV and these topics will be particularly highlighted in this review.

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## 1. INTRODUCTION

Mitochondria are known as "the powerhouse of the cell" due to their vital ability to generate adenosine triphosphate (ATP), which is essential to virtually all cellular processes. Oxidative phosphorylation (OXPHOS) involves the transfer of electrons through the mitochondrial electron transport chain (mETC) and uptake of molecular oxygen resulting in the generation of ATP. Electrons can leak from the mETC react with molecular oxygen generating superoxide radicals, one of several subspecies of reactive oxygen species (ROS). Therefore, mitochondria are also a major source of ROS. Mitochondria have many other roles aside from ATP generation; for example, they play an important role in calcium buffering within cells. Mitochondria also have their own separate DNA—mitochondrial DNA (mtDNA) of which only 5% is not also encoded in the nuclear genome.

Despite extensive research into the contribution of mitochondrial function in other neurological disorders such as Parkinson's disease and Alzheimer's disease, the role of mitochondrial function in sensory processing and pain has been relatively unexplored until the last decade. This chapter will detail research articles that have examined the role of mitochondria in sensory transduction with particular focus on those reports investigating the contribution of mitochondrial function/dysfunction to chronic pain states. As this area of pain research is in its infancy, this chapter will be a descriptive summary, rather than a critical review, of the diverse data published so far that links mitochondrial function/dysfunction as a causal pain mechanism. The wealth of research articles investigating the role of mitochondria in neurodegeneration and apoptosis, which may have some implications in degenerative chronic pain states, will not be described here. This chapter will describe data examining (a) the role of mitochondria in normal sensory processing and acute pain, (b) evidence for mitochondrial dysfunction from patients with painful conditions, and (c) evidence for mitochondrial dysfunction in animal models of chronic pain. In each of these sections, different aspects of mitochondria and their function will be described according to the literature published to date. Such aspects will include mitochondrial ultrastructure, distribution, oxygen consumption, OXPHOS, calcium buffering, ROS, and ATP levels. The experimental techniques utilized to assess these different aspects will also be briefly described.

## 2. ROLE OF MITOCHONDRIA IN NORMAL SENSORY PROCESSING AND ACUTE PAIN

ATP demand varies substantially between different cell types, due to their different functions. Mitochondria are therefore distributed accordingly to meet such variation in ATP requirements. In general, neurons have a reasonably high energetic demand overall. However, when the total size of the neuron—cell body and length of processes—is considered along with the constantly changing activity of different parts of the neuron, energy demand will be variable between structures within any one neuron. Therefore, a common assumption is that mitochondria are not randomly distributed within a neuron and their distribution will change in response to ATP requirements. Most of the primary evidence to support this comes from work examining mitochondrial function in central nervous system (CNS) neurons particularly in the brain. Quantitative analysis of mitochondria in the neuropil of the primate visual cortex demonstrated that over 60% of the total number of mitochondria and mitochondrial area were found in the dendrites.[1] Estimates of the signaling-related energy usage in the gray matter of the rat brain also showed that the predicted energy usage of axons and terminals (~42%) was less than the predicted energy usage of dendrites and soma (~52%).[2] Therefore, despite the greater volume of axons compared to the somatodendritic compartment, they contain less mitochondria and require less energy.

Less work has been reported on mitochondrial distribution and function in peripheral neurons, but there are reports relevant to the role of mitochondria in normal sensory processing. Using electron microscopy and tomographic reconstruction, the 3D structure of mitochondria in axons and Schwann cells in spinal roots of normal mice were described.[3] Condensed mitochondria with dilated cristae comprise roughly two-thirds of axonal spinal root mitochondria, with orthodox mitochondria constituting the remainder. Condensed mitochondria were present in higher concentrations in the nodal region. In comparison, the mitochondria in Schwann cells have a classical orthodox structure only.[3] Studies have examined the distribution of mitochondria and mitochondrial activity in human peripheral sensory nerves finding marked differences between myelinated and unmyelinated axons. Lehmann *et al.* determined the distribution of mitochondria within sensory nerve fibers of sural nerve specimens from humans and primates. In human sural nerves, approximately 55% of mitochondria are found in

myelinated axons, 30% in unmyelinated axons, and 15% in Schwann cells. Similarly, in primates, the majority of mitochondria were found in myelinated axons; approximately 70%, 25%, and 5% of mitochondria were found in myelinated axons, unmyelinated axons, and Schwann cells, respectively.[4] However, when the number of mitochondria was calculated per axonal volume, these authors report that unmyelinated axons have "almost twice" the number of mitochondria compared to myelinated axons. Similar differences have also been observed in the human optic nerve, low cytochrome *c* activity was found in myelinated axons, and high cytochrome *c* activity was found in unmyelinated axons.[5] These findings suggest that unmyelinated axons have a higher energy requirement compared to myelinated axons. Animal studies confirm such a suggestion. Using brain white matter from a variety of different animal species, ranging from shrew to macaque, Wang and colleagues have estimated that unmyelinated axons consume 2.5- to 10-fold more energy per action potential evoked compared to myelinated axons.[6]

Studies of local glucose utilization have shown that energy metabolism alters relative to neuronal activity in a unidirectional manner.[7] Specifically, electrical stimulation of the sciatic nerve in anesthetized rats resulted in a frequency-dependent linear increase in glucose utilization in the lumbar spinal cord.[8] However, these experiments also showed that glucose utilization was unaltered in the dorsal root ganglia (DRG) by sciatic nerve stimulation.[8] This suggests that functional activity is dictated by glucose utilization in nerve terminals rather than cell bodies. More recently, using *in vivo* confocal imaging of saphenous nerves in anesthetized mice, mitochondrial transport has been examined in sensory axons in relation to neuronal activity.[9] Electrical stimulation of myelinated axons increased mitochondrial transport and the velocity of moving mitochondria. At low-frequency stimulation (1 Hz), mitochondrial movement was increased in both anterograde and retrograde directions. In comparison, at high-frequency stimulation (50 Hz), only anterograde mitochondrial movement and velocity was markedly increased. Sajic *et al.* also showed that with increasing electrical stimulation mitochondria accumulate in the skin, but not the DRG, demonstrating that mitochondria accumulate in distal portions of sensory axons with increasing neuronal activity (see Fig. 1). In myelinated axons of the CNS, there is greater accumulation of mitochondria at nodes of Ranvier and decreased mitochondrial transport during increased axonal electrical activity.[10] However, in myelinated axons of the saphenous nerve, no such nodal accumulation of mitochondria during increased electrical stimulation was observed.[9]

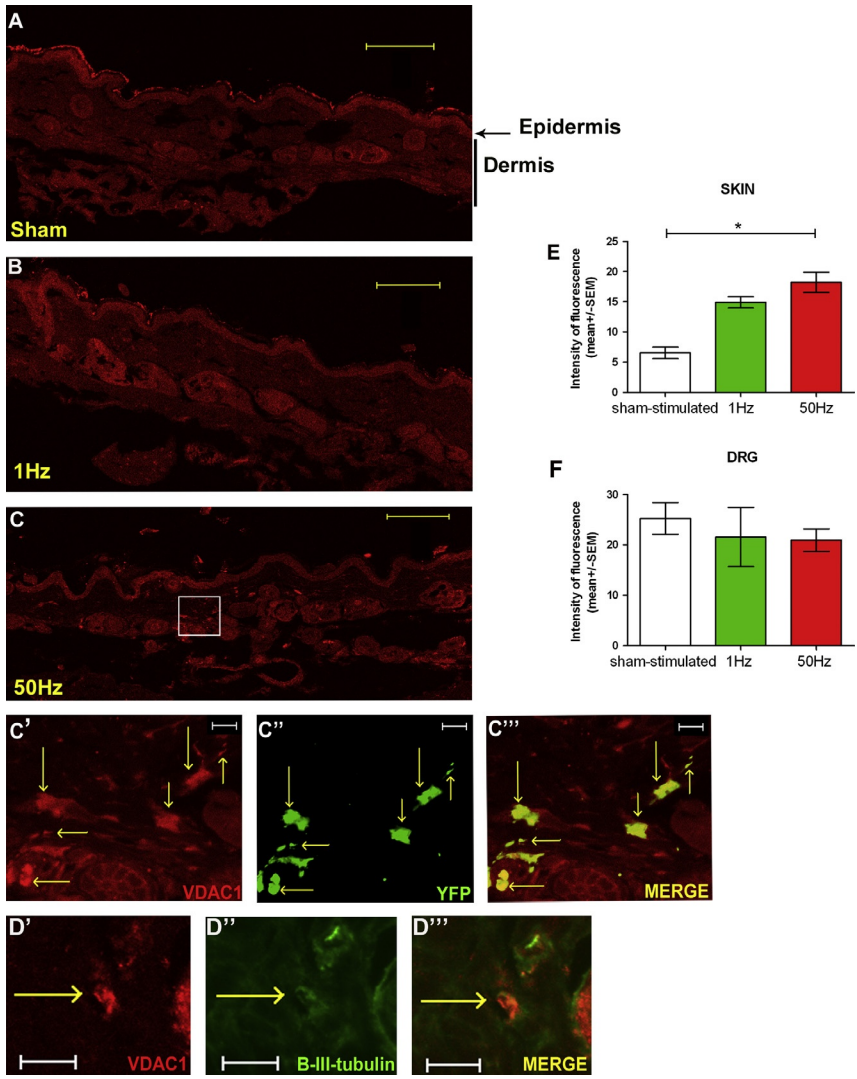


**Figure 1** In normal saphenous nerve axons stimulated at high frequency (50 Hz), mitochondria accumulate at the peripheral sensory terminals. Skin innervated by saphenous nerve was immunohistochemically labeled with VDAC1 (red) and neuron-specific β-III-tubulin (green). In comparison with sham-stimulated animals (A), axons were strongly labeled for VDAC1 in nerves stimulated at 1 Hz (B) and 50 Hz (C). (C′–C‴) Saphenous nerve from a $YFP^{+}$ mouse (shown in low power in C), which expresses YFP (green) in a proportion of fibers, was stimulated with 50 Hz and labeled with VDAC1 (red). (D′–D‴) Saphenous nerve from a $YFP^{-}$ mouse was stimulated with 50 Hz and double labeled with VDAC1 (red) and β-III-tubulin. The two markers were

*(Continued)*

These differences could be due to different methodologies or reflect differences in mitochondrial transport in CNS versus peripheral axons. Using capsaicin application to the skin to directly activate unmyelinated axons, there was a threefold increase in the number of moving mitochondria, but not their velocity, in isolectin B4-positive C-fibers.[9] These effects were observed 15 min following capsaicin application, and this temporally correlates with capsaicin-evoked firing of C-nociceptors,[11] again linking mitochondrial trafficking to neuronal activity in sensory afferents.

Mitochondria play an essential role in calcium buffering within cells, and their importance in the control of intracellular calcium, $[Ca^{2+}]_i$, in sensory neurons has been elegantly demonstrated by the work of Yuriy Usachev's laboratory.[12,13] Using a coculture of DRG and spinal cord neurons to model the first sensory synapse, cytosolic and mitochondrial $Ca^{2+}$ in DRG neurons following capsaicin application were measured in conjunction with excitatory postsynaptic currents (EPSCs) in spinal cord neurons. In a series of experiments, these authors demonstrated that following capsaicin application, $Ca^{2+}$ enters the presynaptic bouton through TRPV1 channels causing a large increase in presynaptic $[Ca^{2+}]_i$ which triggered glutamate release and EPSCs. $Ca^{2+}$ is rapidly taken up by mitochondria in the presynaptic bouton and then gradually transported back into the cytosol via $Na^+/Ca^{2+}$ exchange as opposed to the mitochondrial permeability transition pore (mPTP). This was demonstrated pharmacologically by comparing the effect of CGP37157, a selective inhibitor of mitochondrial $Na^+/Ca^{2+}$ exchange, to cyclosporin A, an mPTP inhibitor. This calcium-buffering mechanism by mitochondria which is coupled to $Ca^{2+}$ efflux via $Na^+/Ca^{2+}$ exchange explains the prolonged elevation in cytosolic $[Ca^{2+}]_i$ in DRG neurons following capsaicin application resulting in further glutamate release and EPSCs. Medvedeva *et al.* also showed that the greater the amount of $Ca^{2+}$ sequestered by mitochondria, the longer the duration of elevated presynaptic $Ca^{2+}$, glutamate release, and postsynaptic activity.[12] Prolonged increases in $[Ca^{2+}]_i$ are also routinely observed in DRG neurons following KCl application. Svichar *et al.* showed that the lipophilic cation tetraphenylphosphonium ($TPP^+$) which blocks both $Na^+$-dependent and $Na^+$-independent mitochondrial

**Figure 1—Cont'd** often found to colocalize. (E) Intensity of VDAC1 labeling was significantly higher within cutaneous fibers of saphenous nerve stimulated at 50 Hz ($n = 5$, $^*p < 0.05$) than in sham-stimulated ($n = 3$) or fibers stimulated at 1 Hz ($n = 3$). (F) There was no difference in VDAC1-labeling intensity in DRGs of saphenous nerves between the groups. Scale bars in (A–C) = 100 μm, in (C′–C‴) = 20 μm, and in (D′–D‴) = 10 μm. *Taken from Ref. 9 figure 3—doi: http://dx.doi.org/10.1371/journal.pbio.1001754.g003.*

$Ca^{2+}$ efflux markedly inhibited KCl-evoked elevations in $[Ca^{2+}]_i$ in both small and large DRG neurons.[14] This further demonstrates the importance of mitochondrial $Na^+/Ca^{2+}$ exchange in presynaptic calcium control in sensory neurons. Further work using cocultures of DRG and spinal cord neurons showed that presynaptic mitochondria also sequestered $Ca^{2+}$ following electrical stimulation.[13] Examining the potential mechanisms of presynaptic $Ca^{2+}$ clearance in capsaicin-sensitive DRG neurons following electrical stimulation showed that presynaptic $Ca^{2+}$ clearance was dependent upon plasma membrane $Ca^{2+}$-ATPase, mitochondria, plasmalemmal $Na^+/Ca^{2+}$ exchange, and sarco–endoplasmic reticulum $Ca^{2+}$-ATPase by approximately 47%, 40%, 12%, and 0%, respectively. Mitochondrial calcium cycling in DRG neurons was also shown to promote the activation of nuclear factor of activated T-cells,[15] which has been implicated in neuronal survival, synaptic plasticity, and axonal growth (discussed in Ref. 15).

The specific effects of mitochondrial calcium uptake on NMDA and capsaicin-evoked pain behaviors and spinal long-term potentiation were assessed using Ru360, a specific inhibitor of the mitochondrial calcium uniporter (MCU).[16] Pretreatment with intrathecal Ru360 significantly inhibited the development of NMDA-evoked mechanical hypersensitivity in the hind paw. However, Ru360 had no effect on established NMDA-evoked mechanical hypersensitivity. NMDA evoked a 2.5-fold increase in mitochondrial $Ca^{2+}$ levels (visualized with Rhod2/AM) in the superficial and deep dorsal horn which was markedly inhibited by pretreatment, but not posttreatment, with Ru360.[16] Ru360 also inhibited NMDA-evoked increases in mitochondrial superoxide levels in the superficial and deep dorsal horn. Using spinal cord slice preparations, Ru360 was shown to block spinal LTP despite large increases in cytosolic $Ca^{2+}$ levels (which were unaffected by Ru360).[16] This indicates that high levels of $Ca^{2+}$ cannot directly trigger LTP unless $Ca^{2+}$ is first sequestered by mitochondria. Blockade of mitochondrial $Ca^{2+}$ uptake also prevented the phosphorylation of several protein kinases in the spinal cord that have been implicated in synaptic plasticity and pain processing, namely protein kinase C, protein kinase A (PKA), and extracellular signal-related kinase (ERK).[16] In recent years, different components of mitochondrial $Ca^{2+}$ transport have been identified in major breakthroughs for the field of mitochondrial research.[17–19] Using RT-PCR of RNA extracted from neuron-enriched DRG cultures, transcripts of the MCU, mitochondrial calcium uptake 1, $Na^+/Ca^{2+}/Li^+$ exchanger, and leucine zipper EF hand-containing transmembrane protein (Letm1) were all observed, providing further evidence for mitochondrial calcium buffering in sensory neurons.[13]

The measurement and manipulation of ROS levels have been performed under both normal conditions and acute pain conditions. In normal mice, intrathecal administration of rotenone (complex I inhibitor) produced a significant mechanical hypersensitivity from 5 h postinjection lasting until 48 h postinjection.[20] In comparison, systemic administration of rotenone had no effect on withdrawal responses to mechanical stimulation in normal rats.[21] Pretreatment with intrathecal rotenone markedly inhibited both NMDA- and capsaicin-evoked mechanical hypersensitivity in mice.[16] Rotenone also prevented NMDA-evoked increases in mitochondrial superoxide levels in the superficial and deep dorsal horn.[16] Intrathecal administration of antimycin A in normal mice also elicited a significant mechanical hypersensitivity from 3 h postinjection lasting until 24 h postinjection.[20] Antimycin A-evoked mechanical hypersensitivity was also shown to be reversible by systemic administration of ROS scavengers.[20] Significant increases in superoxide levels (visualized with MitoSOX) in both the superficial and deep dorsal horn were evident 9 h following intrathecal antimycin A.[20] Intraplantar capsaicin evokes an acute pain syndrome, evidenced with behavioral primary and secondary hyperalgesias that emerge and resolve within 24 h.[22,23] The development and maintenance of capsaicin-evoked secondary hyperalgesia were shown to be ROS-mediated, as ROS scavengers could alleviate capsaicin-evoked secondary, but not primary, hyperalgesia.[22] Capsaicin also increased mitochondrial superoxide levels in the superficial and deep dorsal horn.[22,23] In further work by the same group, superoxide dismutase (SOD-2) activity in the spinal cord was decreased following intraplantar capsaicin.[23] Mice overexpressing SOD-2 did not develop capsaicin-evoked secondary hyperalgesia nor show increased levels of mitochondrial superoxide in the dorsal horn.[23] In contrast, SOD-2 knockout mice developed enhanced capsaicin-evoked primary and secondary hyperalgesias and showed larger increases in mitochondrial superoxide levels, particularly in the deep dorsal horn (laminae III–V).[23] Mitochondrial superoxide has also been implicated in the modulation of neuronal excitability of amygdala neurons and amygdala-mediated behavior via an mGluR5-$IP_3$-ROS-ERK/PKA signaling cascade.[24]

## 3. CLINICAL EVIDENCE OF MITOCHONDRIAL DYSFUNCTION IN PAINFUL CONDITIONS

There is a plethora of inherited mitochondrial diseases, some of which are associated with neuropathy as a diagnostic criterion. The prevalence of

pain caused by mitochondrial disease is not well reported compared to motor consequences of the neuropathies caused by these devastating disorders. Discussion of mitochondrial abnormalities in the context of peripheral neuropathies in mitochondriopathies, per se, is outside the scope of this chapter (for recent review on this topic see Ref. 25). There is debate as to whether polyneuropathy seen in mitochondriopathy is primarily due to mitochondriopathy or a consequence of simultaneously occurring diabetes for example. In a study of 108 patients with mitochondriopathy and neuropathy, the neuropathy in 38 of these patients was found to be only attributable to the mitochondriopathy itself.[26] Of those 38 patients, sensory fibers, motor fibers, and both sensory and motor fibers were predominantly affected in 3, 14, and 21 patients, respectively.[26] Paresthesia, dysesthesia, and hypesthesia were reported in 4, 4, and 10 of these 38 patients, respectively. Muscle ache was reported in 11 of these 38 patients, but no other details of pain symptoms were reported.[26] Detailed descriptive analysis of the changes to the ultrastructure of neuronal mitochondria in sural nerve biopsies from patients with mitochondrial myopathy, hereditary, and nonhereditary neuropathies has also been reported and compared.[27] Charcot–Marie–Tooth neuropathy type 2A, an hereditary axonal peripheral sensorimotor neuropathy that predominantly affects the ankles and feet, is caused by missense mutations in mitochondrial fusion protein mitofusin 2 (MFN2)—a key protein that regulates the mitochondrial network architecture by fusion of mitochondria.[28]

Two case reports describe morphological examination of sural nerve biopsies from patients with chemotherapy-induced neuropathy evoked by paclitaxel[29] and docetaxel.[30] Although not specifically described in these reports, examination of the electron micrographs presented demonstrates the occurrence of swollen, vacuolated mitochondria in sensory axons. Sural nerve biopsies from seven patients with adult-onset diabetes and progressive worsening of their associated neuropathy contained swollen, vacuolated mitochondria in myelinating Schwann cells.[31] In comparison, the mitochondria in nonmyelinating Schwann cells appeared normal; however, glycogen accumulations in the outer compartment of axonal mitochondria (often known as glycogenosomes) were occasionally observed.[31] HIV and one of its treatments have also been found to elicit effects on mitochondria in sensory axons.[32] Sural nerve biopsies were taken from three different groups of patients: HIV patients treated with 2′3′-dideoxycytidine (ddC), HIV patients with neuropathy but without ddC treatment (HIV+), and HIV patients with non-HIV-related axonal polyneuropathy (HIV−).

The percentage of abnormal (swollen and vacuolated) mitochondria found in both myelinated and unmyelinated axons and Schwann cells was then quantified. Approximately 55%, 28%, and 9% of mitochondria in the axoplasm were abnormal in ddC, HIV+, and HIV− patients, respectively. In addition, approximately 47%, 19%, and 14% of mitochondria in Schwann cells were abnormal in ddC, HIV+, and HIV− patients, respectively.[32] These authors also reported a significant decrease in mtDNA in sural nerves from ddC patients compared to HIV− controls. This demonstrates that ddC-induced painful neuropathy (and HIV-induced neuropathy to a lesser extent) can be correlated with a significant increase in abnormal neuronal mitochondria in sensory axons. Lehmann *et al.* compared human DRG and sural nerves postmortem specimens from HIV patients with painful neuropathy (HIV-PN), HIV patients without painful neuropathy (HIV+), and people without HIV (control), for the levels of mtDNA deletion mutations and mitochondrial proteins.[4] Levels of the common $mtDNA^{4977}$ deletion mutation (4977 is indicative of 4977 base pairs)—a marker of mitochondrial damage—were significantly increased in sural nerves from the HIV-PN group compared to HIV+ and controls. However, levels of $mtDNA^{4977}$ deletion in the DRG were similar between the three groups.[4] Further analysis of the sural nerve samples revealed that there was a marked decrease in a mitochondrial glycoprotein in both HIV-PN and HIV+ groups.[4] Moreover, the ratio of mitochondrial glycoprotein to the catalytic subunit I of complex IV (COX-I) was only decreased in the HIV-PN group.[4] This suggests that during HIV painful neuropathy, there is an increase in mtDNA damage and a loss of mitochondria in distal sensory axons.

Skin biopsies from 32 patients with painful sensory distal polyneuropathy caused by various etiologies were double-stained with PGP9.5 and complex IV antibodies, to quantify the number of intraepidermal nerve fibers (IENFs) and mitochondria (OXPHOS-immunoreactivity, OXPHOS-ir), respectively.[33] Based on their IENF density, the biopsies were split into two categories—definite small fiber neuropathy (SFN definite) and SFN borderline—and compared to biopsies from control subjects without neuropathy (control). In biopsies from control subjects without neuropathy, OXPHOS-ir colocalized with IENFs and there was a positive correlation between OXPHOS-ir mitochondria and PGP-positive axons.[33] However, this colocalization and correlation were lost in both the SFN definite and SFN borderline biopsies suggesting that the loss of mitochondria in cutaneous afferents is an early sign of painful peripheral neuropathy.[33]

There is also some clinical evidence suggesting that mitochondrial dysfunction could be contributory to intervertebral disk degeneration and back pain.[34] Cultured annulus cells from human disks of varying degenerative stages (II–V on Thompson Grade) were assessed for mitochondrial morphology, oxygen consumption, and citrate synthase content. There was some descriptive observation of morphological changes, for example, the presence of "abnormally shaped branching mitochondria with small cristae." Both basal respiration and mean mitochondrial respiration were significantly higher in pooled cells from more degenerate Grade III–V disks compared to cells from Grade II disks. Citrate synthase activity (which can considered as a measure of mitochondrial mass) significantly decreased with increasing degree of disk degeneration.[34]

## 4. PRECLINICAL EVIDENCE OF MITOCHONDRIAL DYSFUNCTION IN CHRONIC PAIN STATES

In recent years, data exploring the mechanistic contribution of mitochondrial function to the genesis and maintenance of chronic pain have started to emerge. Such data have mainly arrived from investigations using animal models of chemotherapy-induced, HIV-associated, and diabetic neuropathies. Studies have examined not only mitochondrial function/dysfunction, per se, but also the consequences of mitochondrial dysfunction in terms of the generation of ROS leading to oxidative stress/damage. The following sections will divide this literature in terms of reports detailing the evidence for mitochondrial dysfunction and oxidative stress in different preclinical chronic pain states.

### 4.1 Chemotherapy-induced painful neuropathy

Several widely used first-line chemotherapeutic drugs are associated with painful peripheral neuropathy. This side effect is dose dependent and often dose limiting as there are no treatments to prevent the development of chemotherapy-induced painful neuropathy (CIPN). In addition, many analgesics have failed to show efficacy in patients with CIPN. Chemotherapeutics that are associated with painful neuropathy include taxanes, e.g., paclitaxel; vinca alkaloids, e.g., vincristine; and platinum drugs, e.g., oxaliplatin, epothilones, bortezomib, and thalidomide. For reviews on chemotherapy-induced neuropathies from clinical viewpoints, see Refs. 35,36.

The first evidence that mitochondrial dysfunction is associated with CIPN came from an extensive electron microscopy study comparing

saphenous nerves from paclitaxel- and vehicle-treated rats.[37] Saphenous nerves were compared at three key time points in the paclitaxel-induced pain syndrome: Day 7, 24 h after the last of four systemic injections of paclitaxel—prior to pain onset, day 27—at the peak of paclitaxel-induced pain behavior, and day 160—at the resolution of paclitaxel-induced pain behavior. Atypical neuronal mitochondria were identified as vacuolated (>50%) and/or markedly swollen and were present in C-fibers and myelinated axons of both paclitaxel- and vehicle-treated rats (Fig. 2). The striking findings of this study were a 64% and 128% increase in the prevalence of atypical mitochondria in C-fibers of paclitaxel-treated rats at day 7 and day 27, respectively. Furthermore, similar findings were observed in myelinated axons, with a 137% and 159% increase in the prevalence of atypical mitochondria day 7 and day 27, respectively. In contrast, at day 160, there were no significant changes in neuronal mitochondria in C-fibers or myelinated axons. Therefore, these paclitaxel-induced changes in neuronal mitochondria could be correlated to the development and maintenance of paclitaxel-induced pain syndrome, i.e., present prior to and during paclitaxel-induced pain, but absent when the pain syndrome had resolved. These low-dose paclitaxel-induced mitochondrial changes in C-fibers and myelinated axons have been further confirmed in other reports.[38–40] Further investigation using electron microscopy identified increased incidence of swollen/vacuolated mitochondria in C-fibers and A-fibers of the dorsal root at the peak of paclitaxel-induced pain behavior, but no change in the A-fibers of the ventral root.[39] No changes in the incidence of atypical mitochondria were observed in the Schwann cells (myelinating and nonmyelinating) of the saphenous nerve, dorsal, or ventral roots.[39]

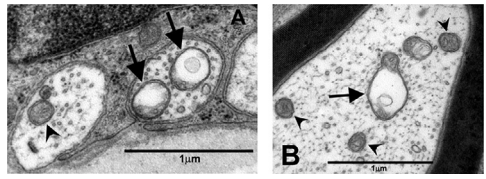


**Figure 2** Examples of atypical neuronal mitochondria found in the saphenous nerve of paclitaxel-treated rats at the peak of paclitaxel-induced pain behavior. (A) C-fibers and (B) small, thinly myelinated Aδ-fiber from a paclitaxel-treated rat at day 27 posttreatment initiation. NB: Atypical mitochondria were observed in both small and large diameter myelinated axons. Magnification: 44,400×. *Taken from Ref. 37, figures 9 and 10. The figures have been reproduced with permission of the International Association for the Study of Pain® (IASP). The figures may NOT be reproduced for any other purpose without permission.*

Swollen/vacuolated mitochondria were also observed in DRG neuronal cell bodies following low-dose paclitaxel.[41] Collectively, these data show that only mitochondria in sensory axons are susceptible to paclitaxel perhaps providing some explanation as to why paclitaxel produces a predominantly sensory neuropathy. Swollen/vacuolated mitochondria have also been observed in saphenous nerves from rat models of oxaliplatin-induced painful neuropathy[42] and bortezomib-induced painful neuropathy.[43] In both studies, nerves were only examined at the peak of the oxaliplatin-/bortezomib-induced pain behaviors (~day 35 after first injection of oxaliplatin/bortezomib) and found significant increases in atypical axonal mitochondria in C-fibers and A-fibers. Similar to paclitaxel, there was also no significant change in the mitochondria in myelinating and nonmyelinating Schwann cells of saphenous nerves from oxaliplatin- and bortezomib-treated rats.[42,43]

Although the identification of swollen/vacuolated neuronal mitochondria provides evidence for mitochondrial dysfunction, it does not indicate the nature of the mitochondrial dysfunction that has occurred. Assays of mitochondrial function are required to decipher what aspects of mitochondrial function have been compromised and their consequences. Using a Clark-type oxygen electrode with high-resolution respirometry (Oroboros Oxygraph-2k), oxygen consumption was measured from sciatic nerve preparations from paclitaxel-, oxaliplatin-, and bortezomib-treated rats.[43,44] There were significant decreases in complex I-stimulated and complex II-stimulated respiration in nerves from paclitaxel-, oxaliplatin-, and bortezomib-treated rats compared to nerves from vehicle-treated rats. These effects were observed prior to chemotherapy-induced pain onset and at the peak of chemotherapy-induced pain behavior.[43,44] Using oxygen and pH-sensing fluorophores in a multiwell plate format (XF24 Extracellular Flux Analyzer), we have simultaneously measured both oxygen consumption and glycolysis in DRG cells from paclitaxel- and vehicle-treated rats.[45] By pharmacologically modulating the mETC, we measured basal respiration and glycolysis, ATP-linked respiration, maximal respiration, spare reserve capacity, and glycolytic capacity. There was a significant decrease in maximal respiration and spare reserve capacity (the ability of mitochondria to respond to stress) in DRG cells from paclitaxel-treated rats compared to DRG cells from vehicle-treated rats at day 7 prior to the emergence of pain behavior. At the peak of paclitaxel-induced pain behavior, there was a significant increase in basal glycolysis and glycolytic capacity in DRG cells from paclitaxel-treated rats. At the resolution of paclitaxel-induced pain behavior,

there was no difference in oxygen consumption or glycolysis measures between paclitaxel- and vehicle-treated rats.[45]

Mitochondria play a fundamental role in the generation of ATP through OXPHOS. Basal ATP levels in sciatic nerves from paclitaxel-, oxaliplatin-, and bortezomib-treated rats were unaltered compared to those from vehicle-treated rats.[43,44] However, there was a reduction in the amount of ATP generated following stimulation (addition of complexes I and II substrates plus ADP) in sciatic nerves from paclitaxel-, oxaliplatin-, and bortezomib-treated rats relative to those from vehicle-treated rats. These effects were also observed prior to and at the peak of chemotherapy-induced pain behavior.[43,44] The ATP content (or lack of) within a cell, per se, is not necessarily indicative of mitochondrial (dys)function, as extensively discussed elsewhere.[46] The adenine nucleotide pool and its equilibrium should also be considered.[46] ATP synthase (complex V) function can be reversed, so less observed ATP could be accompanied with increased ADP, for example. We have observed decreases in ATP, but not ADP, levels in DRG from paclitaxel-treated rats compared to vehicle-treated rats at the peak of paclitaxel-induced pain behavior.[45]

There are a variety of pharmacological tools that can selectively inhibit/modulate different aspects of the ETC and mitochondrial function. In a series of experiments, rats with vincristine-induced mechanical hyperalgesia received intradermal administration of mETC inhibitors for complexes I–V.[47] All inhibitors produced modest reduction in mechanical thresholds over a 3-h period. The magnitude of reduction observed was between approximately 15–38% and was most pronounced at 30 min postadministration of all inhibitors. The smallest effect was observed with rotenone (complex I inhibitor) and the largest effect with sodium cyanide (complex IV inhibitor).[47] In other studies by a different group, 30 min following systemic rotenone, there was approximately 20–25% enhancement of the mechanical hypersensitivity induced by paclitaxel and oxaliplatin.[21] Paclitaxel- and oxaliplatin-induced mechanical hypersensitivity was also increased by 20–35%, 30 min following systemic oligomycin (an inhibitor of ATP synthase). Oligomycin also increased discharge frequency in A-fibers and C-fibers (to a larger extent) in both paclitaxel- and oxaliplatin-treated rats. These facilitatory effects were seen from 5 min postinjection, peaked at 15–20 min and were close to preinjection levels again by 30 min.[21] We have shown the causal role of ROS in chemotherapy-induced pain behaviors (discussed below). Complexes I and III are sources of electron leak from the mETC resulting in ROS generation. Therefore, we have

administered rotenone (complex I inhibitor) and antimycin A (complex III inhibitor) in acute and prophylactic dosing paradigms in paclitaxel-treated rats to assess the effects of complex I and III inhibition on the maintenance and development of paclitaxel-induced pain.[48] We found that rotenone and antimycin A inhibited paclitaxel-induced mechanical hypersensitivity 1 h following administration. In addition, we found that prophylactic antimycin A attenuated the development of paclitaxel-induced mechanical hypersensitivity, whereas rotenone had no effect. In summary, these behavioral studies have shown that direct modulation of the mETC *in vivo* can have various consequences on chemotherapy-induced pain behaviors. The differences in observed effects may be explained by route of drug administration, inhibitor in question or time point examined post administration.

Other pharmacological studies in rodents displaying chemotherapy-induced pain behaviors have examined the role of ROS *in vivo*. Phenyl *N-tert*-butylnitrone (PBN), a nonspecific ROS scavenger, reversed established paclitaxel-induced mechanical hypersensitivity[49,50] and paclitaxel-induced cold allodynia.[50] PBN also markedly inhibited the development of paclitaxel-induced mechanical hypersensitivity, when administered in a prophylactic paradigm which that encompasses the week immediately following paclitaxel administration.[49,50] 4-Hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL), an SOD-2 mimetic, inhibited established paclitaxel-induced mechanical hypersensitivity at higher doses than PBN but was ineffective on cold allodynia and the development of paclitaxel-induced mechanical hypersensitivity.[50] Peroxynitrite decomposition catalysts have been shown to reverse established paclitaxel-induced mechanical hypersensitivity[51] and to also prevent the development of mechanical hypersensitivity induced by paclitaxel, oxaliplatin, and bortezomib.[51,52] Recently, an increase in ROS and reactive nitrogen species (RNS) levels was seen in lumbar DRG following chronic oxaliplatin treatment in mice.[53] SS-31, a novel mitochondria-targeted antioxidant, reversed this oxaliplatin-evoked ROS/RNS increase and also attenuated oxaliplatin-induced cold and mechanical hypersensitivities.[53] Collectively, these data suggest that mitochondrial ROS is causal to the development and maintenance of (CIPN). Acetyl-L-carnitine (ALC) is involved in free fatty acid oxidation and also acts as an antioxidant. Initial studies demonstrated that prophylactic ALC administration could prevent the development of paclitaxel-induced mechanical hypersensitivity.[54] Closer analysis of the saphenous nerves from these animals showed that ALC had prevented the paclitaxel-evoked increase in swollen/vacuolated mitochondria in

C-fibers.[38] Prophylactic ALC administration was also reported to prevent paclitaxel-, oxaliplatin-, and bortezomib-evoked compromises in complex I-stimulated and complex II-stimulated respiration in sciatic nerves.[43,44] Despite promising reports of ALC efficacy from preclinical studies[54–57] and an open-label phase II trial of CIPN patients,[58] a recent placebo-controlled randomized clinical trial did not support the use of ALC as a treatment for CIPN.[59] Breast cancer patients received daily doses of ALC or placebo for 24 weeks from the beginning of their paclitaxel chemotherapy. At 12 weeks, there was no difference between ALC and placebo; however, at 24 weeks, CIPN and functional status of patients who had received ALC had significantly worsened.[59]

As discussed previously, there are a variety of pharmacological agents that specifically modulate different complexes of the mETC. There are other pharmacological reagents that directly interact with mitochondria and their function in different ways. Two such reagents have been evaluated for their effects on chemotherapy-induced pain behaviors.[60–62] Cholest-4-en-3-one, Oxime (TRO19622/Olesoxime), is a cholesterol-like compound that directly binds to two components of the mPTP: the voltage-dependent anion channel and the translocator protein 18 kDa (or peripheral benzodiazepine receptor).[63] Olesoxime had no effect on mechanical thresholds in normal rats.[60] In contrast, olesoxime produced ~50% reversal of established vincristine- and paclitaxel-induced mechanical hypersensitivity.[60,61] Olesoxime also attenuated both the development of paclitaxel-induced mechanical hypersensitivity and loss of IENFs. However, olesoxime had no effect on paclitaxel-induced spontaneous discharge in C- and A-fibers.[61] Another pharmacological reagent that interacts with a different aspect of mitochondrial function is mdivi-1, which is a selective Drp1 inhibitor. Dynamin-related protein 1 (Drp1) is a GTPase which catalyzes mitochondrial fission (the process of mitochondria dividing to increase mitochondria numbers). Intradermal injections of mdivi-1 significantly attenuated oxaliplatin-induced mechanical hyperalgesia.[62]

## 4.2 Diabetic painful neuropathy

Diabetic polyneuropathy is the most common complication associated with Type I and Type II diabetes that has been estimated to affect around two-thirds of patients in their lifetime. Diabetic polyneuropathy is a "dying-back," length-dependent neuropathy characterized by a loss of neurons which begins with the most distal axons in the feet and progresses up the

legs. Diabetic polyneuropathy causes ongoing pain, sensory loss, foot ulceration, poor wound healing, and infection potentially leading to gangrene and amputation. Poor glucose control was thought to be the key factor in the genesis of diabetic polyneuropathy; however, it has been recently highlighted that glucose control has a large effect on prevention of neuropathy caused by Type I diabetes, but only a small effect on prevention of neuropathy caused by Type II diabetes.[64]

The majority of preclinical research into diabetic painful neuropathy has used chemically induced diabetes evoked by a single injection of streptozotocin (STZ), although there are many other models of diabetes (for review, see Ref. 65). STZ causes rapid ablation of pancreatic beta cells resulting in a loss of insulin production and hyperglycemia. STZ-treated rodents have been reported to display both mechanical and thermal hyperalgesia and hypoalgesia, with hyperalgesia behaviors often preceding hypoalgesia behaviors.[66,67] Different pharmacological tools which directly interact with mitochondria and modulate their function have been assessed for their impact on STZ-evoked mechanical hypersensitivity. Intradermal administration of mETC inhibitors for complexes I–V reduced STZ-evoked mechanical hyperalgesia by approximately 12–35%, 30 min following administration.[47] The smallest reduction was obtained by rotenone and the largest reduction by sodium cyanide.[47] Oral administration of olesoxime (TRO19622) inhibited STZ-evoked mechanical hypersensitivity following acute and repeated administration and also inhibited STZ-evoked heat hyperalgesia.[60] Olesoxime also significantly reduced STZ-evoked slowing of compound muscle action potentials, but had no effect on the sensory nerve conduction velocities.[60] The effects of ROS scavenging compounds have also been evaluated in STZ-evoked behaviors. *N*-Acetylcysteine inhibited thermal hyperalgesia and improved motor coordination in STZ rats.[68] Peroxynitrite decomposition catalysts (Fe(III) tetramesitylporphyrin octasulfonate—FeTMPS and Fe(III) tetrakis-2-(*N*-triethylene glycol monomethyl ether)pyridyl porphyrin—FP15) attenuated mechanical hypoalgesia, heat hypoalgesia, and mechanical hyperalgesia, but had no significant effect on mechanical allodynia in STZ mice.[69,70] Alleviation of both sciatic motor nerve conduction velocities and hind-limb digital sensory nerve conduction velocities was also observed following treatment with FeTMPS.[70] An array of biochemical changes associated with diabetic neuropathy occurs in STZ-treated rodents. Increased lipid peroxidation in sciatic nerves and increases in nitrotyrosine levels in the sciatic nerve, DRG, and spinal cord are reported.[68–70] Decreases in phospholipids,

$Na^+$–$K^+$-ATPase activity, thiols (nonprotein—GSH and protein), and antioxidant enzyme (SOD-2, catalase, glutathione peroxidase, glutathione reductase, glutathione-*S*-transferase) activity levels were all observed in sciatic nerves of STZ rats.[68] *N*-Acetylcysteine significantly corrected all of these biochemical changes except nitrotyrosine levels (which was not examined).[68] FeTMPS and FP15 treatment significantly reduced STZ-evoked increases in nitrotyrosine levels in the sciatic nerve, DRG, and spinal cord.[69,70]

Studies assessing neuronal mitochondria and mitochondrial dysfunction in preclinical models of diabetic neuropathy have examined several different aspects including ultrastructure, mitochondrial membrane potential, oxygen consumption, mitochondrial proteome, mitochondrial ROS, and mitochondrial calcium levels. Mitochondrial ultrastructure has been examined in several models of Type I diabetes. In diabetic BioBreeding/Worcester rats, there was no morphological evidence of mitochondrial abnormality in the DRG.[71] In nonobese diabetic and STZ-induced diabetic mice, "swollen neurites containing nearly pure collections of mitochondria" were observed in superior mesenteric–celiac ganglia.[72] In the spontaneously genetic diabetic Akita mouse (another model of Type I diabetes), tiny hyperchromatic dense mitochondria were found in the prevertebral sympathetic ganglia.[73] In comparison, in a model of Type II diabetes (db/db mice) when diabetic neuropathy had developed, there was an increase in the number of mitochondria in both myelinated and unmyelinated axons of the dorsal root.[74] Interestingly, the number of mitochondria in the ventral root did not change in db/db mice at the same time point or in younger diabetic mice prior to the emergence of diabetic neuropathy.[74] Enhanced mitochondrial biogenesis and fission have also been indicated in sensory neurons in db/db mice by increases in mtDNA and mitochondrial density and decreased mitochondrial diameter.[75]

The mitochondrial membrane potential ($\Delta\psi_m$) can be estimated by loading cells with a fluorescent probe (e.g., rhodamine 123, TMRM) and then applying an ionophore uncoupler, such as FCCP, to collapse $\Delta\psi_m$. The ratio between the fluorescent value prior to FCCP and following FCCP application is directly proportional to the status of $\Delta\psi_m$. DRG neurons isolated from STZ rats and loaded with rhodamine 123 showed that there was a loss in $\Delta\psi_m$ indicating that the mitochondrial membrane potential was depolarized during diabetic neuropathy.[76,77] Using TMRM, there was also evidence that neuronal mitochondria were depolarized in the axons of cultured DRG neurons from STZ rats.[78] Using the Oroboros Oxygraph-2k,

oxygen consumption was measured from lumbar DRG tissue homogenates and mitochondria isolated from lumbar DRG of STZ rats.[79] Although basal respiration was unaffected, significant deficits in coupled OXPHOS and complex IV-mediated respiration were found in lumbar DRG tissue homogenates of STZ rats. Similarly, isolated mitochondria from 22-week-old STZ rats also showed deficits in coupled OXPHOS, uncoupled respiration, and complex IV-mediated respiration, whereas basal respiration was normal.[79] In further work, using the XF24 Extracellular Flux Analyzer, this group also demonstrated marked deficits in maximal respiration and spare reserve capacity in intact DRG neurons isolated from 22-week-old STZ rats compared to age-matched controls.[80] Recently, deficits in maximal respiration and spare reserve capacity of sensory neurons have also been associated with sensory hypoalgesia evoked by Type II diabetes.[81] Interestingly, these authors also report that STZ-evoked sensory hypoalgesia in mice precedes the deficit in spare reserve capacity of sensory neurons suggesting that mitochondrial dysfunction in more closely correlated with diabetic neuropathy evoked by Type II diabetes than Type I diabetes.[81] The deficits in mitochondrial membrane potential and oxygen consumption in DRG neurons from STZ rats could be a consequence of decreased expression and activity of mETC components. Protein levels and enzymatic activity levels of complexes I and IV, but not complex V, were decreased in the DRG of STZ rats compared to controls.[79,80] Citrate synthase activity and PGC-1α (considered a major regulator of mitochondrial biogenesis and cellular energy metabolism) expression were also significantly decreased in DRG from STZ rats.[79,80] An extensive proteomic analysis of DRG from 22-week-old STZ rats revealed that proteins associated with OXPHOS and mitochondrial dysfunction, e.g., subunits of complexes I, IV, and V, and SOD-2 were significantly downregulated.[78]

ROS levels in sensory neurons from diabetic rodents have also been examined with different fluorescent probes.[74,78,79] ROS levels—assessed by both DHR 123 and DCF fluorescence—were similar in acutely isolated DRG neurons from STZ and control rats.[79] Hydrogen peroxide levels (detected with Amplex Red) were also similar in isolated mitochondria from the DRG of STZ and control rats.[79] Axons of DRG cultures from STZ rats showed increased levels of DCF fluorescence and 4-HNE adducts, but significant decreases in mitochondrial superoxide levels—imaged with MitoSOX Red.[78] The increased ROS found in axons from STZ cultures was attributed to activity of the polyol pathway (also known as the sorbitol–aldose reductase pathway—an alternative pathway by which

unused glucose is metabolized), as opposed to deficient mETC function.[78] DRG cultures from db/db mice showed increased mitochondrial superoxide levels in neurites and also in neuronal cell bodies following an hour's exposure to high glucose.[74] Finally, there is some evidence[82] showing altered mitochondrial calcium buffering in small DRG neurons of STZ and db/db mice, which could contribute to deregulated calcium homeostasis in diabetic neuropathy (for reviews on this topic, see Refs. 83,84).

## 4.3 HIV-associated painful neuropathy

HIV-associated painful neuropathy originates from two aspects—the HIV infection itself and the antiretroviral drugs used to control HIV. Regardless of the specific origin patients present with the same neuropathic pain symptoms including ongoing pain and mechanical allodynia/hyperalgesia in both feet.[85] HIV-associated painful neuropathy is a typical "dying-back," length-dependent neuropathy, with symptoms starting in the toes and then progressing up the legs. Once at mid-leg level, symptoms may then also start in the fingertips.[85]

Similar to observations from HIV patient samples, sural nerves from macaques infected with simian immunodeficiency virus (SIV) showed reductions in mitochondrial glycoprotein and the mitochondrial glycoprotein:COX-I ratio compared to controls.[4] Additional experiments using isolated mitochondria from sural and sciatic nerves of SIV-infected, highly active antiretroviral therapy (HAART)-treated, and control macaques provided further evidence of mitochondrial dysfunction in sensory neurons.[4] Increased susceptibility to mitochondrial permeability, increased hydrogen peroxide production, and a loss of mitochondrial membrane potential were observed in the mitochondria from sural, but not sciatic, nerves of SIV-infected and HAART-treated macaques.[4] In addition, sural nerves from SIV-infected macaques showed increased oxidative stress evidenced by increased carbonyl groups and nitrotyrosine levels.[4]

Nucleoside analog reverse-transcriptase inhibitors (NRTIs) were some of the first drugs used to control HIV and provide the backbone of combination HAART regimens. 2′-3′-Dideoxycytidine (zalcitabine), 2′,3′-dideoxyinosine (ddI—didanosine), and 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T—stavudine) are all NRTIs and are particularly associated with painful peripheral neuropathy. There are only a few reports using animal models of HIV-treatment-associated painful neuropathy that explore the potential connection of mitochondrial (dys)function to this

disorder. Rats with ddC-induced mechanical hyperalgesia received intradermal administration of mETC inhibitors for complexes I–V[47] in separate experiments. Similar to the effects seen in vincristine-treated rats, mechanical hyperalgesia in ddC-treated rats was reduced by approximately 15–38% and was most pronounced at 30 min postadministration of all inhibitors. The smallest reduction was elicited by rotenone and the largest reduction by sodium cyanide.[47] Intrathecal administration of antisense against Drp1 reversed ddC-evoked mechanical hyperalgesia.[62] In addition, intradermal administration of mdivi-1, a selective Drp1 inhibitor, also reversed ddC-evoked mechanical hyperalgesia.[62] The effects of feline immunodeficiency virus (FIV) and ddI, when given alone and in combination, on mitochondrial COX-I levels in DRG neurons were compared under *in vitro* and *in vivo* conditions.[86] *In vitro*, only combined FIV and ddI exposure significantly reduced COX-I mRNA, but not DNA, levels.[86] *In vivo*, FIV and ddI significantly decreased expression of COX-I DNA and protein levels.[86] No further decrease in COX-I DNA and protein levels was seen in DRGs from FIV-infected cats treated with ddI, although such additive effects were seen in COX-I mRNA levels.[86] Two intravenous injections of d4T (on days 0 and 4, in the absence of immunodeficiency virus) produced evoked and spontaneous pain behaviors in rats.[87] Proteomics on sural nerves harvested at day 7 found 10 proteins that were downregulated with at least 10-fold changes in d4T animals.[87] Three of these proteins were related to mitochondrial function, namely mitochondrial long-chain specific acyl-CoA dehydrogenase, NADH dehydrogenase (ubiquinone) 1 alpha subcomplex subunit 4, and mitochondrial ATP synthase subunit D.[87] Further analysis of sural nerves with Western blotting showed a 50% reduction of mitochondrial long-chain-specific acyl-CoA dehydrogenase following d4T.[87]

## 4.4 Other chronic pain states

Neuropathic pain and persistent inflammatory pain are typically modeled in rodents using peripheral nerve trauma via surgical techniques and Complete Freund's Adjuvant (CFA) injection into the hind paw, respectively. Using the spared nerve injury (SNI) and CFA mice models, the numbers of mitochondria in the dorsal horn were visualized following intrathecal administration of a fluorescent mitochondrial marker.[88] Seven days following CFA and 14 days following SNI, there were significant increases in the number of mitochondria in laminae I–V of the ipsilateral side of the spinal cord compared to the contralateral side.[88] Up to day 7 following partial sciatic

nerve ligation (PSNL), an increase in cytochrome *c* levels in the spinal cord was reported[89] indicating mitochondrial dysfunction as cytochrome *c* is normally contained within the inner and outer mitochondrial membranes. Furthermore, systemic administration of cyclosporin A, an mPTP blocker, pre- and post-PSNL inhibited PSNL-evoked mechanical allodynia.[89]

The majority of the studies that suggest a potential role of mitochondrial dysfunction in neuropathic and inflammatory pain have focussed on ROS and oxidative stress. CCI-evoked heat hyperalgesia could be inhibited by antioxidants TEMPOL,[90] *N*-acetylcysteine,[91] and tirilazad.[92] In addition, increased SOD-2 levels were found in the axotomized sciatic nerve.[93] PBN, a nonspecific ROS scavenger, inhibited mechanical hypersensitivity evoked by spinal nerve ligation,[94–96] capsaicin-induced inflammation,[22,97] and visceral inflammation.[98] Other nonspecific ROS scavengers, 5,5-dimethylpyrroline-*N*-oxide and nitrosobenzene also relieved neuropathic pain behaviors.[94] Reagents that mimic SOD-2 activity inhibited hypersensitivity to mechanical/heat stimuli evoked by either peripheral nerve injury[90,96] or inflammation.[22,97,99,100] Finally, mitochondrial ROS-producing profiles, visualized through *in vivo* delivery of a fluorescent mitochondrial marker, were increased in the spinal cord following peripheral nerve injury[101] or an inflammatory stimulus.[22,23]

## 5. FUTURE PERSPECTIVES

In this chapter, I have summarized the evidence that suggests a contributory role of mitochondrial function to normal sensory processing and chronic pain. This particular area of research is novel to the pain research field with most studies only dating back over the last decade or so. The majority of these investigations have highlighted evidence for a specific contributory/causal role of mitochondria in painful peripheral neuropathies evoked by chemotherapy, diabetes, and HIV. The functions of mitochondria are diverse, and at present, there are many questions remaining as to how these different mitochondrial functions are contributory to pain states and also how different mitochondrial functions are modulated and/or impaired during chronic pain conditions. There are also several possibilities as to how dysfunctional mitochondria may mechanistically cause pain via, for example, ATP deficiency, and/or excessive ROS, and/or impaired calcium buffering. Further research using a range of assays of mitochondrial function will elucidate these mechanisms and aid analgesic drug development. Although modulating mitochondrial function could potentially have

knock-on detrimental effects on cellular respiration, selective and careful targeting of mitochondrial function may well yield novel analgesics. Further research will also identify if mitochondrial modulation could be beneficial to chronic pain disorders of various etiologies or specifically to drug-induced neuropathies such as CIPN.

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