
Copyright: Copyright © 2013 the authors.

This article is licensed under a Creative Commons Attribution-Noncommercial-Share Alike 3.0 Unported license.

DOI link to article: http://dx.doi.org/10.1523/JNEUROSCI.3525-12.2013

Date deposited: 24th July 2014
Mitochondrial defects within substantia nigra (SN) neurons are implicated in the pathogenesis of Parkinson’s disease. SN neurons show increased mitochondrial defects, mitochondrial DNA deletion levels, and susceptibility to such dysfunction, although the role of mitochondria in neuronal degeneration remains uncertain. In this study, we addressed this important question by exploring changes within the mitochondria of SN neurons from patients with primary mitochondrial diseases to determine whether mitochondrial dysfunction leads directly to neuronal cell loss. We counted the pigmented neurons and quantified mitochondrial respiratory activity, deficiencies in mitochondrial proteins, and the percentage of pathogenic mutations in single neurons. We found evidence of defects of both complex I and complex IV of the respiratory chain in all patients. We found that marked neuronal cell loss was only observed in a few patients with mitochondrial disease and that all these patients had mutations in polymerase gamma (POLG), which leads to the formation of multiple mitochondrial DNA deletions over time, similar to aging and Parkinson’s disease. Interestingly, we detected α-synuclein pathology in two mitochondrial patients with POLG mutations. Our observations highlight the complex relationship between mitochondrial dysfunction and the susceptibility of SN neurons to degeneration and α-synuclein pathology. Our finding that the loss of SN neurons was only severe in patients with POLG mutations suggests that acquired mitochondrial defects may be less well tolerated by SN neurons than by inherited ones.

**Introduction**

Mitochondrial dysfunction is thought to play a role in several different neurodegenerative diseases, including Parkinson’s disease (PD) and other synucleinopathies (Abou-Sleiman et al., 2006; Schapira, 2007; Surmeier et al., 2011; Vives-Bauza and Przedborski, 2011). Several explanations for the increased sensitivity of substantia nigra (SN) neurons to mitochondrial dysfunction have been proposed, including increased levels of iron and calcium metabolism within this brain region. A role for this dysfunction in the pathogenesis of PD has been strengthened over the past few years by the discovery that several of the genes responsible for early onset familial forms of these diseases have a role associated with mitochondrial function, including Pink1 (PARK6 [MIM 608309]), parkin (PARK2 [MIM 602544]; Narendra et al., 2008, 2010), and DJ-1 (PARK7 [MIM 602533]; Mutsu and Nakagawa, 2001), whereas α-synuclein (PARK1 [MIM 168601]) is thought to damage mitochondria directly (Devli et al., 2008; Parihar et al., 2008). In addition, high levels of respiratory deficiency and mitochondrial DNA (mtDNA) deletions are found within the neurons of the SN both in patients with PD and in normal aging (Bender et al., 2006; Kratskberg et al., 2006). mtDNA mutations have also been implicated as being important for PD based upon cybrid studies (Swedlow et al., 1996; Gu et al., 1998). Previous studies have shown reduced activity of mitochondrial complex I in SN neurons and PD-like symptoms in response to various drugs and toxins, including MPTP and rotenone (Burns et al., 1983; Langston et al., 1983; Schapira et al., 1998). Despite evidence suggesting a role for mitochondrial dysfunction in the loss of SN neurons, there remains considerable uncertainty about the mechanisms involved.

Mutations within mtDNA also cause a group of diseases termed the mitochondrial disorders, which have a minimum prevalence of clinical manifestation in 1 in 5000 people (Schaefer et al., 2008). Neurological defects including ataxia, seizures, dementia, and stroke-like episodes are common in patients with...
Reeve et al. • Pathogenic mtDNA Mutations and SN Neurons

mtDNA deletions, and five patients with autosomal recessive mutations encompassed patients harboring different mtDNA mutations with different syndromes, including two patients with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) due to the m.3243A→G mutation, two patients with myoclonic epilepsy with ragged red fibers (MERRF) due to the m.8344A→G mutation, two patients with Kearns–Sayre syndrome (KSS) due to single large-scale mtDNA deletions, and five patients with autosomal recessive mutations in POLG with multiple mtDNA deletions. Table 2 shows a summary of control patients used in this study, including age, sex, post mortem delay, cause of death, and histopathological features in the SN.

Tissue
All midbrain tissue for this study (with the exception of single deletion case 2; Shanske et al., 1990) was obtained from the NBTR. The collection and use of all human tissue in this study was approved by the appropriate local research ethics committee and conformed to the United Kingdom Medical Research Council’s guidelines on the use of tissue in medical research. The midbrain tissue from mitochondrial disease cases was matched to an appropriate control. Patients ranged in age from 20 to 79 years (mean, 46.2) and included 6 females and 5 males. Table 1 shows relevant clinical data for all of the mitochondrial patients used within this study. Control subjects ranged in age from 48 to 70 years (mean, 56) and included 7 males and 5 females. Details of these control subjects, including the cause of death, can be found in Table 2. An age-matched control was found for the majority of patients; however, finding exact matches to the younger mitochondrial disease cases proved more difficult.

Histology and immunohistochemistry
With the exception of the second single large-scale mtDNA deletion case (single deletion 2), all tissue was treated identically. Due to the limited number of single large-scale deletion cases available within the NBTR, we were limited in the available tissue sections for this second single deletion case. Two 20-μm-thick sections of formalin-fixed paraffin-embedded upper midbrain were taken from each case at the start and end of the cutting series. These thick sections were stained using cresyl fast violet (CFV) and were used for neuronal counting. Immunohistochemistry for five mitochondrial proteins (porin, complex II [succinate dehydrogenase]) 70 kDa subunit, complex I (NADH:CoQ oxidoreductase) 19 kDa subunit, complex I 20 kDa (ClO2) subunit, and complex IV (cytochrome c oxidase [COX]) subunit I (Mitosciences), α-synuclein (Novacastra), and tyrosine hydroxylase (TH; Sigma) was performed on 5 μm formalin-fixed paraffin-embedded sections, as described previously (Lax et al., 2012a; Reeve et al., 2012) on tissue from 10 cases, including single deletion 2. The cells within the SN were then counted as described below.

Materials and Methods
Mitochondrial disease patients
A total of 11 patients with clinically and neuropathologically confirmed mitochondrial disease were included. All patients were severely affected with mitochondrial disease and some had been previously included in a study examining the pathology of cerebellar ataxia in patients with mitochondrial disease (Table 1 and Lax et al., 2012a). Due to the limited number of single large-scale deletion patients within the Newcastle Brain Tissue Resource (NBTR), we acquired sections from a patient with a single large-scale mtDNA deletion from Dr K. Tanji (Columbia University, New York, NY). Details of this patient have been described previously (Shanske et al., 1990).

Table 1 shows neurological features of these 11 patients and the presence of any extrapyramidal features that were present, as well as details of mutations within both mitochondrial and nuclear genes. This study

![Table 1. Summary of the patient cohort used for this study including age, sex, genotype, major neurological features, the presence of extrapyramidal involvement and neuropathology of the SN](image-url)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sex</th>
<th>Genotype</th>
<th>Major neurological features</th>
<th>SN pathology</th>
<th>Presence of extrapyramidal involvement</th>
<th>Patient number</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Female</td>
<td>m.3243A→G</td>
<td>Ataxia, stroke-like episodes, cognitive impairment, deafness, encephalopathy</td>
<td>Normal neuronal density; pale neuromelanin in pigmented cells; α-Syn/LB: none</td>
<td>No</td>
<td>Patient 6</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>Female</td>
<td>m.3243A→G</td>
<td>Ataxia, stroke-like episodes, epilepsy, dementia, deafness, encephalopathy, migraine, depression</td>
<td>Moderate loss of neurons in LNB, less pronounced in the UMB; α-Syn/LB: none</td>
<td>No</td>
<td>Patient 1</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>Female</td>
<td>m.8344A→G</td>
<td>Ataxia, deafness, myopathy, myoclonus, depression</td>
<td>Intact neuronal population</td>
<td>No</td>
<td>Patient 8</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>Male</td>
<td>m.8344A→G</td>
<td>Ataxia, epilepsy, peripheral neuropathy, myoclonus, ataxia</td>
<td>Intact neuronal population; mild to moderate vacuolation; α-Syn/LB: none</td>
<td>No</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Female</td>
<td>m.11756-15636</td>
<td>Ataxia, dementia, encephalopathy, depression, myopathy, CPEO, heart block</td>
<td>Intact neuronal population; mild to moderate vacuolation; α-Syn/LB: none</td>
<td>No</td>
<td>Patient 11</td>
<td></td>
</tr>
</tbody>
</table>
Deficient cells were those with an absence of immunoreactivity for the mitochondrial protein of interest. From the patients from whom frozen material was available (controls 7, 8, 11, and 12 and all mitochondrial disease cases with the exception of POLG1 and 2), 5 μm sections were stained for activity in complexes IV (COX) and II (succinate dehydrogenase [SDH]), using the previously reported COX/SDH assay (Betts et al., 2006).

**Neuronal cell counts in SN**

The extent of nigral neuron loss in the 10 patients with mitochondrial disease and the 10 age-matched controls (obtained from the NBTR) could not be determined from dissector counts in serial sections of the whole nigral nucleus because these were unavailable. Instead, a counting method was devised based on the findings of Ma et al. (1995a, 1995b) that dissector counts show good correlation with single two-dimensional dissector counts and that also contained neuromelanin (i.e., the SN). The SN was outlined at the appropriate rostrocaudal level within the control where serial sections were available. The neuron count was then calculated as a percentage of the neuron count at the same level within this control, allowing the absolute cell number to be determined. Cells with a visible so that we did not remove matter from the neuropil. Single cells were cut into 500 μm thick CFV-, Loyez-, and hematoxylin/eosin-stained sections (Table 1). The rostrocaudal levels of the SN were defined from 16 levels of serially cut 20-μm-thick CFV- and hematoxylin/eosin-stained sections of upper and lower midbrain from one normal control patient 74 years of age. The definitions of the levels were based on relative positions and shape of myelin fiber tracts, including cranial nerve III, medial longitudinal, fasciculus, medial lemniscus and its subdivisions, and deccascation of brachium conjunctivum in relation to the dorsal and ventral tereus of the SN.

For the second single large-scale mtDNA deletion patient, only 5 μm sections were available. The cell counts could not be compared in the same manner as above because the orientation of the section did not allow comparison with any of the levels in the control. Therefore, the density of the neurons within the SN was calculated based on the area of the SN and compared with the density of neurons within a similar 5 μm section from the first single mtDNA deletion case and controls.

**Determination of percentage level mutated mtDNA to wild-type mtDNA**

Sample preparation for molecular analysis. For both point mutation and deletion level analysis, cryostat sections of upper midbrain (20 μm thick) were mounted onto PEN-membrane slides (Leica Microsystems) and COX/SDH histochemistry was performed. Single COX-positive neurons were dissected from the SN, collected, and lysed (LMD 6000; Leica Microsystems). Briefly, cells to be dissected are drawn around in the software and the laser then cuts out the individual cell. To limit contamination, we took care to cut as close to the cell boundary as possible so that we did not remove matter from the neuropil. Single cells were cut into 500 μl tubes and the tube caps inspected to verify the presence of only one cell per tube. To limit the risk of contamination, neurons were cut into tubes that had been UV sterilized before each session of cutting. DNA extraction was performed at 55°C using a standard lysis buffer for a minimum of 3 h, with a proteinase K inactivation session of cutting. DNA extraction was performed at 55°C using a standard lysis buffer for a minimum of 3 h, with a proteinase K inactivation session of cutting.

Pyrosequencing for point mutations. The quantification of mtDNA mutation load in single SN neurons was performed as described previously (Lax et al., 2012a). For this study, two pyrosequencing assays were used: one for m.3243A>G and the other for the m.8344A>G mutation. The quantification of heteroplasmic levels for mtDNA point mutations has been validated previously (White et al., 2005). Pyrosequencing was performed as per the manufacturer’s protocol (Qiagen) using a mutation-specific pyrosequencing primer. Pyromark Q24 software (Qiagen) was used to quantify mutated mtDNA heteroplasmy levels by comparing directly the relevant peak heights of both wild-type and mutant mtDNA at this site.

**Real-time PCR for mtDNA deletions**. A multiplex real-time PCR MT-ND1/MT-ND4 assay was used to quantify the levels of mtDNA deletions in individual SN neurons, as described previously (Krisnan et al., 2007; Lax et al., 2012a). This assay detects deletions that occur within the major arc of mtDNA, and the majority of mtDNA deletions are thought to occur within this region in both disease and aging (Samuels et al., 2004). A total of 2 μl of sample was used and run in triplicate in a 96-well plate. Each run also included known deletion-level standards and a blood control, also run in triplicate.

**Statistical analysis**. An unpaired t test was used to compare mutation loads between patients and controls.

---

**Table 1. Continued**

<table>
<thead>
<tr>
<th>Single deletion 2</th>
<th>POLG 1</th>
<th>POLG 2</th>
<th>POLG 3</th>
<th>POLG 4</th>
<th>POLG 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 14</td>
<td>Patient 12</td>
<td>Patient 13</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

| Patient 14 | Patient 12 | Patient 13 | N/A    | N/A    |

α-Syn, Synuclein; LB, Lewy body; LMB, lower midbrain; UMB, upper midbrain; CPEO, chronic progressive external ophthalmoplegia.

α-Syn/LB: none

Moderately severe neuronal loss; α-Syn/LB: moderate

Severe loss of UMB SN neurons; α-Syn/LB: none

No

No

Parkinsonian symptoms

No

No

N/A subject

From Lax et al. (2012a,b).

Reported in Shanske et al. (1990).
Results

Lewy body pathology

All cases and controls used in this study were stained for α-synuclein (for clinical and pathological data, please refer to Tables 1 and 2). The presence of α-synuclein immunoreactive Lewy bodies, pre-Lewy bodies, and cytoplasmic granular inclusions in pigmented SN neurons was used as evidence of Lewy body pathology. None of the controls showed any immunoreactivity for α-synuclein within the SN. α-Synuclein immunoreactivity was also absent from the m.8344A>G MERRF, m.3243A>G MELAS, and KSS single large-scale mtDNA deletion cases despite the potential effects that mitochondrial dysfunction could have on the ubiquitin proteasome system (for review, see Livnat-Levanon and Glickman, 2011). α-Synuclein-positive Lewy body pathology was, however, found in two of the five POLG cases (POLG 3 and 4) used in this study, one of which has been described previously (Betts-Henderson et al., 2009; Fig. 1). Lewy body pathology in these two cases included Lewy bodies and neurites plus cytoplasmic granular staining of SN neurons. It is particularly interesting that POLG patient 4 showed little cell loss above that associated with normal aging and yet showed severe α-synuclein pathology.

Deficiency of mitochondrial proteins in SN neurons

In this study, we examined the levels of 5 mitochondrial proteins within SN neurons in 10 patients with mitochondrial disease and 8 controls (POLG 1 and 2 of the controls were excluded from this analysis due to an extremely long fixation time severely affecting immunohistochemistry). We examined two subunits of complex I of the electron transport chain [complex I 19 kDa (CI19) and complex I 20 kDa (CI20)] because deficiencies of this protein have been reported in patients with mtDNA disease and in both elderly individuals and PD cases (Schapira et al., 1989; Distelmaier et al., 2009; Lax et al., 2012a; Reeve et al., 2012). We also examined levels of complex IV subunit I (COXI), which has been reported previously to be correlated with activity in this complex (Mahad et al., 2009). Complex II (CI170) was also studied because it is fully encoded by the nuclear genome and preserved in the presence of mtDNA disease. To assess changes in mitochondrial density within SN neurons, we used porin, a protein on the mitochondrial outer membrane. For complexes I and IV, the number of SN neurons within a 5 μm section with clearly defined nuclei were counted and the number of deficient cells calculated.

Porin and CI170 immunohistochemistry did not reveal any difference in mitochondrial density within SN neurons between patients with mitochondrial disease and controls (Fig. 2). There were no neurons that showed a deficiency of complex II or porin.
mtDNA deletions were present in control neurons (mean for 3 cases, in single neurons. Consistent with previous reports, we found that neurons, we examined the overall heteroplasmy levels of mutations To examine the effect of the pathogenic mtDNA mutations on SN mitochondrial disorders mtDNA mutation levels are high in the SN of patients with mtDNA deletion patients exhibited the lowest levels of COXI deficiency at 0.9% and 2.7%, respectively.

Neurons with deficiencies of complex IV activity are more common in patients with POLG mutations For some cases, frozen midbrain tissue was available, allowing not only the study of mutation loads but also a comparison between the immunohistochemistry and the activity of complex IV using COX/SDH histochemistry. This assay allows the detection of cells with defects in the activity of complex IV (Fig. 5). The results from this assay replicated those from the immunohistochemistry. Controls and patients with point mutations showed low levels of COX deficiency (Fig. 5E, patterned columns; controls, 5.5 ± 4.5%; MELAS1, 1.9%; MELAS2, 1.3%; MERRF1, 7.6%; and MERRF2, 10.8%), whereas patients harboring POLG mutations and multiple mtDNA deletions showed the highest levels (20.7 ± 7.1%). Single deletion patient 1 exhibited very low levels of COX deficiency at 0.15%, which equated to the presence of only one COX deficient neuron shown in Figure 5.

mtDNA mutation levels are high in the SN of patients with mitochondrial disorders To examine the effect of the pathogenic mtDNA mutations on SN neurons, we examined the overall heteroplasmy levels of mutations in single neurons. Consistent with previous reports, we found that mtDNA deletions were present in control neurons (mean for 3 cases, 25.6 ± 4.2%; Fig. 6A) and that the percentage level of these deletions rarely reached >60% (Bender et al., 2006; Kraysberg et al., 2006).

mtDNA deletion levels within the POLG and single large-scale mtDNA deletion patients (CI20, 13.75%; CI19, 8.27%). For COX1 (Figs. 4, 5E), controls again showed the lowest levels of deficiencies (4.2 ± 2.2% range, 1.1–8.0%), with cases MELAS1 (4.08%) and MERRF2 (3.18%) falling within this range. MELAS2 had a slightly higher level of deficiency (8.23%), followed by tissue from MERRF2 (22.9%). The patients with POLG deficiency and multiple deletions showed high levels of deficiency for this protein (14.8 ± 5.9%). Interestingly, the single mtDNA deletion patients exhibited the lowest levels of COXI deficiency at 0.9% and 2.7%, respectively.

The percentage levels of pathogenic point mutations were also measured using mutation specific pyrosequencing assays. High percentage levels of mutation were found in the majority of single neurons (Fig. 6B), with a mean mutation load of >80% for both the m.3243A>G (MELAS) and the m.8344A>G (MERRF) patients. The heteroplasmy levels for both mtDNA deletions and point mutations within SN neurons of these patients are comparable to the levels recently described within the cerebellar neurons of patients with mtDNA disease (Table 3; Lax et al., 2012a), an area of the brain where extensive cell loss is associated with respiratory deficiencies caused by the pathogenic mtDNA mutation.

In COX-positive normal neurons within the SN, we found levels of mtDNA mutations comparable to those seen within the cerebellar neurons (Table 3). It is interesting that these mutation levels were associated with cell loss in both the cerebellum and the SN in the patient POLG3, but in the patients with inherited defects, the levels of mtDNA mutation within the SN comparable to levels within the cerebellum were not associated with cell loss.

Neuronal loss within the SN of patients with mtDNA disorders For each patient with mitochondrial disease and each control used for this study, 20 μm (with the exception of single deletion 2), CFV-stained sections of midbrain were counted us-
Figure 3. Complex I immunohistochemistry showing the variable degree of protein deficiency in SN neurons. Pigmented SN neurons with a deficiency in complex I subunits (arrows) were found in all cases and controls; however, the level of deficiency varied. A–H, Images show staining for CI20 (A–D) and CI19 (E–H), and the arrows indicate neurons showing deficiencies for these proteins. (Figure legend continues.)
ing the stereological microscope and the average of the two counts obtained. Although there was some variation among individuals, the mean neuron count from 10 controls expressed as a percentage of the count from the corresponding rostro-caudal level of SN from the normal serially sectioned midbrain was 96 ± 31% (Fig. 7). This variation would be expected based on the variability between controls from other studies (Fearnley and Lees, 1991). In addition, age-related loss of SN neurons is known to occur in normal individuals at a rate of 4.7% per decade (Fearnley and Lees, 1991), although the age range of the control cases used in this study was relatively narrow (48–70 years; mean, 58 ± 6.9 years; n = 10). The availability of tissue from younger individuals was much lower than for the elderly individuals, so finding an appropriate match for MELAS 1 and POLG 2 was particularly difficult. MELAS patients who harbored the m.3243A>G pathogenic point mutation showed variable changes, with one patient showing moderate loss of the SN neurons and the other showing a greater number of SN neurons than controls, whereas both m.8344A>G MERRF patients showed moderate cell loss (Fig. 7). There was evidence of only mild SN neuron loss in the patient with KSS due to a single large-scale deletion despite the very high levels of complex I deficiency (COXI protein levels and COX activity levels were normal). Although we could not use the same method to examine neuronal loss in the second single deletion patient because we had only thin sections, the density of pigmented neurons within the SN (10.35 cells per mm²) was comparable to that because we had only thin sections, the density of pigmented neurons within the SN (10.35 cells per mm²) was comparable to that of the other single deletion patient 1 (7.96 cells per mm²). This suggested that, in both cases, the cell loss was minimal compared with controls (mean cell density, 8.49 ± 2.53 cells per mm² for 6 control cases). The most severe SN neuron loss was seen in three of the five POLG cases (POLG 2, POLG 3, and POLG 5), in whom SN neuron counts were <30% of controls and more comparable to the SN neuron loss seen in patients with PD/dementia with Lewy bodies (DLB; Fig. 7A). This neuron loss is particularly dramatic when the age of the patients is taken into consideration and could represent an acceleration of the amount of SN neuron loss that occurs with normal aging. However, the moderate neuron loss in POLG 1 and absence of any apparent neuron loss in POLG 4 suggest that more complex factors are involved. In addition, the presence of α-synuclein-positive Lewy body pathology in the SN in two of the five POLG cases, one with severe loss of SN neurons (POLG 3) and the other without any neuron loss (POLG 4), indicates that vulnerability of SN neurons to degeneration and susceptibility to PD/DLB α-synuclein pathology are not simple.

In a number of our patients (8/11, both m.3243A>G MELAS and m.8344A>G MERRF cases, both single deletion cases, POLG 4, POLG 5, and controls 2, 4, and 8), we performed TH staining and found that in all patients except POLG 5, between 80% and 100% of pigmented cells were TH positive. These data also confirm the high correlation between the two methods used for estimating cell loss within the SN. In POLG 5, the percentage of positive cells was only 60%, confirming the severe loss of dopaminergic cells in this case (Fig. 7B).

**Extrapyramidal features in patients with mitochondrial disease**
Data from this study show that cell loss does occur within the SN of some patients with mitochondrial disease, particularly those harboring POLG mutations. Observing extrapyramidal involvement in patients with complex neurological symptoms is difficult. POLG 3 had clear parkinsonian signs and the presence of α-synuclein-positive Lewy bodies (Betts-Henderson et al., 2009) and Parkinsonian symptoms are well documented in patients with POLG mutations (Luoma et al., 2004; Mancuso et al., 2004; Davidzon et al., 2006; Pagnamenta et al., 2006; Tzoulis et al., 2006; Hudson et al., 2007; Galassi et al., 2008; Invernizzi et al., 2008; Remes et al., 2008; Sato et al., 2011). A lack of obvious extrapyramidal features in the patients does not necessarily mean that they were not present or that the loss of neurons within the SN is not significant.
Deficiency in COX activity within SN neurons revealed by COX/SDH histochemistry. Respiratory (COX)-deficient neurons were found in the SN of all patients with mitochondrial disease and controls used within this study. However, the COX-deficient neurons (blue) were sparse in patients with KSS/single large-scale deletion (this patient had only one COX-deficient neuron; A) and point mutations (B). POLG/multiple deletion patients (C) generally showed higher levels of COX deficiency than the patients with inherited defects. Few COX-deficient (Figure legend continues.)
We have shown that the neurons of the SN are affected by mitochondrial dysfunction in patients with a variety of genetically determined primary mitochondrial diseases. The level of respiratory chain deficiency was marked in some patients, but severe neuron loss appeared to be more frequent in patients with mutations in POLG. This is reflected in the clinical observations that Parkinsonism is a feature repeatedly reported in patients with POLG (Luoma et al., 2004; Mancuso et al., 2004; Davidzon et al., 2006; Pagnamenta et al., 2006; Tzoulis et al., 2006; Hudson et al., 2007; Galassi et al., 2008; Invernizzi et al., 2008; Remes et al., 2008; Betts-Henderson et al., 2009; Sato et al., 2011). Deficiency of complex I occurs in many patients, but is most severe in those patients harboring mtDNA deletions. Changes in both the activity and level of complex IV proteins are less pronounced than for complex I, but, again, occur in many patients and are most severe in those with POLG mutations. Similar to other brain regions, complex I deficiency in the SN, while potentially causing cell dysfunction, does not immediately produce marked cell loss. Similar to the cerebellum, the consequences of complex IV deficiency seem to be much less well tolerated and there is likely to be a progression from complex I to complex IV deficiency; it is this more severe deficiency that has major consequences for disease (Lax et al., 2012a).

We believe our findings shed some further light on the pathogenesis of Parkinson’s disease and other synucleinopathies; for example, how the mitochondrial defect and α-synuclein aggregation might interact with each other. We only saw α-synuclein accumulation in two patients, both with POLG mutations, and we did not detect any α-synuclein pathology in the other cases despite marked respiratory chain deficiency in some cases. Therefore, although both mitochondrial defects and α-synuclein are linked to the pathogenesis of PD/DLB, the mitochondrial defect alone is not responsible for the accumulation of α-synuclein. This is entirely compatible with our previous observations in patients with PD/DLB in which the mitochondrial defects were seen in neurons without α-synuclein pathology (Reeve et al., 2012).

The observation that mitochondrial defects are likely to play a role in PD is built upon strong genetic, toxin, and animal model studies. It is also quite striking how some patients with POLG mutations develop clear extrapyramidal features and associated loss of SN neurons, yet such features are not prominent in many other patients with mitochondrial disease. In addition, as seen even in this relatively small cohort of patients, SN involvement was not a universal feature in all patients with POLG mutations. Indeed, one of our patients who had both POLG mutations and fairly extensive α-synuclein pathology had little neuron loss in the SN, confirming the variation seen in mitochondrial disease.
Do SN neurons exposed to mitochondrial dysfunction from early life adapt to survive?

Our data suggest that there may be differences in the severity of SN changes seen in mitochondrial patients depending on whether their mitochondrial defect develops with advancing age (in the case of POLG/multiple deletion patients) or if it is present from early development (single large-scale mtDNA deletion and point mutation patients). Cell loss and moderate levels of deficiency in complex I and complex IV seem to be associated with the age-related accumulation of a defect, whereas very high mutation levels, mitochondrial deficiencies, and relatively mild cell loss are associated with both the single large-scale mtDNA deletion patients and those patients with mtDNA point mutations. In the latter group of patients, the defect had been present since birth, which raises the intriguing hypothesis that SN neurons, which are subjected to mitochondrial dysfunction from an early stage, could have an ability to adapt to mitochondrial dysfunction to survive and maintain function. Those neurons in which a defect develops later in life are less able to adapt and therefore are more susceptible to the dysfunction and might be lost.

Among the possibilities to explain this phenomenon is the possibility of an early switch to glycolysis to maintain cellular function. Studies with trans-mitochondrial cybrids have shown that high levels of mitochondrial dysfunction can cause the cells to reverse ATP synthase to maintain both mitochondrial membrane potential and ATP generation (Abramov et al., 2010) or can switch to glycolysis (Pallotti et al., 2004). A shift to glycolysis and increases in Krebs cycle flux have also been reported in Drosophila in response to mitochondrial dysfunction caused by various mutations (Celotto et al., 2011), whereas changes in protein expression within metabolic pathways have also been shown to occur in PD (Mandel et al., 2005; Zhang et al., 2005; Elstner et al., 2011). These studies show a decrease in oxidative phosphorylation genes, tricarboxylic acid cycle, and glycolytic genes; however, Elstner et al. (2011) showed that the expression of most genes is stable with aging and that decreases occur specifically in PD. Therefore, in patients with inherited pathogenic mtDNA mutations that occur in early development, a switch to a different energy generation could occur, allowing the developing SN neurons to survive despite harboring mitochondrial dysfunction, whereas with aging and in patients with acquired defects, this change does not occur.

Another possibility is a switch in the pacemaker channels over time. The subtle and intriguing balance between calcium handling and mitochondrial function has been studied in depth by Surmeier et al. (Chan et al., 2007; Surmeier, 2007; Surmeier et al., 2011). SN neurons contain an almost unique calcium channel (CaV 1.3 L type) that modulates the pacemaking activity of these neurons (Chan et al., 2009). Under experimental conditions, blocking these calcium channels caused SN neurons to revert to a juvenile form of channel that maintains this pacemaking activity through the movement of sodium ions (Chan et al., 2007). This switch to the juvenile channels also protects the neurons against oxidative stress and rotenone. It is possible that SN neurons that carry mitochondrial dysfunction from early life maintain their juvenile channels and their pacemaking activity through sodium rather than calcium. This would protect them against oxidative dysfunction within the SN, preventing neuron loss. This possibility remains to be investigated.

Conclusions

Mitochondrial dysfunction is common and varied within the SN of patients with mitochondrial disorders. The accumulation of mutations over time may be more detrimental for neuronal survival than the presence of mitochondrial dysfunction throughout life. These findings suggest intriguing possibilities that might unlock new therapeutic avenues to protect cells against mitochondrial dysfunction within this important brain region.
References


Parikh MS, Parihar A, Fujita M, Hashimoto M, Ghafouri-Fard P (2008) Mi-

Copyright © 2010 by Elsevier Ltd. All rights reserved.


