Astrocytic Changes in Mitochondrial Oxidative Phosphorylation Protein Levels in Parkinson’s Disease

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ABSTRACT: Background: Mitochondrial dysfunction within neurons, particularly those of the substantia nigra, has been well characterized in Parkinson’s disease and is considered to be related to the pathogenesis of this disorder. Dysfunction within this important organelle has been suggested to impair neuronal communication and survival; however, the reliance of astrocytes on mitochondria and the impact of their dysfunction on this essential cell type are less well characterized.

Objective: This study aimed to uncover whether astrocytes harbor oxidative phosphorylation (OXPHOS) deficiencies in Parkinson’s disease and whether these deficiencies are more likely to occur in astrocytes closely associated with neurons or those more distant from them.

Methods: Postmortem human brain sections from patients with Parkinson’s disease were subjected to imaging mass cytometry for individual astrocyte analysis of key OXPHOS proteins across all five complexes.

Results: We show the variability in the astrocytic expression of mitochondrial proteins between individuals. In addition, we found that there is evidence of deficiencies in respiratory chain subunit expression within these important glia and changes, particularly in mitochondrial mass, associated with Parkinson’s disease and that are not simply a consequence of advancing age.

Conclusion: Our data show that astrocytes, like neurons, are susceptible to mitochondrial defects and that these could have an impact on their reactivity and ability to support neurons in Parkinson’s disease. © 2021 The Authors. Movement Disorders published by Wiley Periodicals LLC on behalf of International Parkinson Movement Disorder Society.

Key Words: mitochondria; OXPHOS; imaging mass cytometry; Parkinson’s disease

Mitochondrial dysfunction plays a role in the loss of dopaminergic neurons (DNs) in Parkinson’s disease (PD),1,2 although a direct relation with neurodegeneration remains elusive. An important player in the survival of neurons is astrocytes, although it is currently unclear whether astrocyte function is altered in PD and any impact of this on DN survival. Being essential for neuronal function and survival, astrocytes provide metabolic support, transport water, and control/maintain the blood–brain barrier.3 Astrocytes release neurotrophic factors, particularly important for the survival of DNs,4 maintain and affect synaptic transmission, are essential for the recycling of neurotransmitters, and can increase responses to glutamate and γ-aminobutyric acid.5,6 These glia also support the...
development and elimination of synapses and their maturation and population with receptors,7 and interestingly, several familial PD genes have been shown to be critical for astrocyte function.4

Though predominantly characterized by the degeneration of DN within the substantia nigra (SN), neuroinflammation is increasingly recognized as an important part of PD pathogenesis. This inflammatory response includes reactive astrocytes; however, it remains unclear whether this is a primary event in the degenerative process or a consequence of the loss of DN.4,8 6-OHDA - 6 hydroxy dopamine (6-OHDA) has been shown to activate a microglial response that can be modulated by treatment with an anti-inflammatory preventing DN loss.9 Treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine also induces a glial response, which occurs before the degeneration of DN.10,11

Deficiencies in key mitochondrial proteins have been uncovered within DN from the SN.12,13 often caused by the accumulation of mitochondrial DNA (mtDNA) deletions.14,15 Many familial PD genes encode proteins with critical mitochondrial functions12 or the ability to inhibit their function.16,17 Despite the importance of neuronal mitochondrial dysfunction to the pathogenesis of PD, a detailed assessment of mitochondrial oxidative phosphorylation (OXPHOS) function within astrocytes is lacking. Although, astrocytes, unlike neurons, are not thought to rely on OXPHOS for the production of ATP, instead favouring glycolysis18,19, they do produce lactate, considered an important fuel source for neuronal mitochondrial respiration, particularly important for synaptic transmission.20-22 Although the delivery of lactate would be beneficial to healthy neurons, it could be detrimental to neurons with mitochondrial dysfunction, further depleting nicotinamide adenine dinucleotide (NAD+), exacerbating OXPHOS deficiency and producing toxic lactic acid.23 Despite a reliance on glycolysis, OXPHOS defects within astrocytes have been shown to impair their proliferation, increasing neurodegeneration after ischemia. Importantly, astrocyte survival was not affected, suggesting that OXPHOS defects impair the ability of astrocytes to support neurons rather than the basic functions required for their survival.24

Therefore, the assessment of OXPHOS within astrocytes may suggest further hypotheses regarding the development and propagation of neurodegeneration. Here, we studied astrocytes within the SN of individuals with PD and age-matched controls to provide the first detailed description of OXPHOS protein expression in astrocytes in PD.

Materials and Methods

Human Tissue
Formalin-fixed paraffin-embedded sections of postmortem human upper midbrain at the level of the superior colliculus were obtained from the Newcastle Brain Tissue Resource (NBTR, https://nbt.ncl.ac.uk/). Although the obtained sections were not at precisely the same level, astrocytes were sampled from within the SN based on the geographical location and the presence of neuromelanin in neighboring neurons. Written consent had been given by the donors or next of kin, with ethical approval provided by the National Health Service Local Research Ethics Committee, and their use adhered to the Medical Research Council guidelines on the use of human tissue in medical research. Clinically and pathologically confirmed sporadic PD patients (n = 9, mean age: 79.2 ± 6.9 years; age range: 68–84 years; Table 1) were compared to neurological healthy controls (n = 10, mean age 67.9 ± 21.5 years). This cohort was included in our previous neuronal study13 and incorporated 2 young individuals who acted as normal controls (18 and 19 years) and 8 individuals aged-matched with the PD patients (age range: 60–93 years; mean age: 80.2 ± 10.6 years).

Imaging Mass Cytometry

Imaging mass cytometry (IMC) is an antibody-based technique, which allows the simultaneous detection of multiple protein targets (~40) within individual cells, through the use of lanthanide-conjugated antibodies. Each antibody is conjugated to a unique lanthanide tag. Tissue sections labeled with antibodies are laser ablated, and through mass spectrometry individual heavy metals and their proteins of interest can be separated, with abundance recorded. Due to the unique way the tissue is ablated micron by micron, the Hyperion retains spatial information allowing reconstruction of the ablated tissue, with the signal intensity of each target protein logged. Midbrain sections (5 μm) were subjected to an IMC panel comprising 12 conjugated antibodies: including 2 cell markers for DN (tyrosine hydroxylase [TH]) and active astrocytes (glial fibrillary acidic protein, GFAP), 1 nuclear marker (Histone H3), and 9 mitochondrial proteins spanning all OXPHOS complexes. This antibody panel was previously optimized, tested, and validated; the details are available in reference 13 and in Table S1. The GFAP antibody (Agilent Z0334, RRID:AB_10013382, Santa Clara, USA) was used at 0.013 mg/mL and conjugated to heavy metal 141Pr. Antibody labeling of sections for IMC detection was performed as earlier.13,25

Image Analysis

IMC was performed with a Hyperion imaging system coupled to a Helios mass cytometer (Fluidigm, San Francisco, USA) as detailed in references 13 and 26. Generated pseudo-images were exported as tiffs (MCD viewer, Fluidigm) and converted to pseudocolored images for visualization using QuPath 0.2.0.27 Individual astrocytes in the SN were manually segmented based on positive cytoplasmic GFAP signal (cellular
| Case | Sex | Age (y) | Disease duration (y) | PM delay (h) | Fixation time (w) | Clinical presentation | Lewy pathology stage | LB Braak stage | Neuropathology | Number of astrocytes analyzed |
|------|-----|---------|----------------------|-------------|------------------|----------------------|----------------------|----------------|----------------|-------------------------------|
| PD01 | M   | 68      | 9                    | 30          | 5.86             | Presented with tremor in the right arm, bradykinesia, rigidity, and poor balance | Entorhinal I |           | Brain with α-synuclein pathology; allocortical tau pathology; mild iso and allocortical amyloid pathology; mild meningeal CAA in the occipital lobe | 46             |
| PD02 | M   | 70      | 15                   | 48          | 7.57             | PD without dementia; no cognitive impairment | Limbic IV |           | Brain with subcortical α-synuclein pathology and with tau pathology mainly in the allocortex, without any Aβ pathology except for very mild meningeal CAA in the occipital lobe | 82             |
| PD03 | M   | 80      | 5                    | 88          | 14.14            | PD without dementia, pronounced autonomic features particularly postural hypotension | Limbic IV |           | Limbic Lewy body disease with mild allocortical tau pathology | 44             |
| PD04 | M   | 80      | 10                   | 34          | 7.43             | Tremor, dyskinesia, drooling, postural hypotension, vivid nightmares, and visual hallucinations at times | Neocortical VI | | Neocortical Lewy body disease | 48             |
| PD05 | M   | 81      | 12                   | 13          | 9                | Loss of balance, tremor, some bradykinesia at onset | Neocortical VI | | Brain with α-synuclein pathology; neocortical Lewy body disease with additional tau pathology mainly restricted to the limbic system | 43             |
| PD06 | M   | 82      | 11                   | 7           | 4.86             | Cognitively intact 3 months before death; tremor-dominant PD | Neocortical VI | | Lewy body disease; Alzheimer-type pathology restricted to medial temporal lobe | 58             |
| PD07 | M   | 83      | 6                    | 30          | 6.29             | Bilateral tremor with rigidity and bradykinesia, some shuffling, mild postural instability, and mild hypomimia | Brainstem III | | Brainstem type of Lewy body disease; mild allocortical tau pathology restricted to the entorhinal cortex | 29             |
area >20 pixel²; median and interquartile range [IQR]: 135.13 and 76.65 pixel²) and a clear nuclear signal from Histone H3. The signal intensity values per pixel (normalized to the area) of individual astrocytes were measured by Qupath software for each tested mitochondrial protein. A total of 924 astrocytes were analyzed, with a mean of 49 astrocytes per case (Nmin–max = 13–90; mean number for PD, 50.9 ± 14.5; control, 47.7 ± 21.6).

### Statistical Analysis

Statistical analysis was performed using R 3.6.1. Original intensity values from IMC for individual astrocytes were natural-logged transformed, and z scores were generated. z Scores for each mitochondrial complex protein were calculated based on their regression to the mitochondrial mass (VDAC1/porin) level of the control data set, which represents the protein expression level normalized to mitochondrial mass per individual astrocyte. Z_VDAC1 was generated from the number of standard deviations away from the mean of the control expression a datapoint was (http://mito.ncl.ac.uk/immuno/29). A statistical decrease in the expression of each targeted protein was determined when plotted data points fell below the lower limit of the 80% predicted interval of the control linear regression against mitochondrial mass (VDAC1). Statistical differences between groups were described using Bayesian estimation, Mann-Whitney U test, and Wilcoxon signed-rank tests, alongside linear mixed effects modeling, to account for the impact of potential variants between individuals (the difference in the number of cells analyzed) within each group. Moreover, multiple linear regression with interaction modeling was used to determine the effects of age and disease/disease duration on protein expression.

### Results

Mitochondrial dysfunction within SN neurons is well characterized in PD, though the mitochondrial health of astrocytes is not established. Thus, to provide in-depth characterization of mitochondrial OXPHOS protein expression within astrocytes in this brain region, we utilized IMC to analyze GFAP-positive astrocytes. We recognize that although this does not allow the capture of all astrocytes, GFAP is an astrocyte marker, which is upregulated in the context of neurodegeneration and should label reactive astrocytes. Representative images from a control (Con09) and PD (PD07) astrocyte demonstrate decreased signal for a number of mitochondrial proteins within the PD case compared to intact signals in the control (Fig. 1A).
FIG. 1. Legend on next page.
Individual Variability in Astrocytic OXPHOS Protein Expression

To eliminate the impact of variations in mitochondrial mass on the evaluation of OXPHOS deficiency within each individual astrocyte, we generated a z score representation of the relative expression of OXPHOS proteins normalized to mitochondrial mass. The median z score of each targeted OXPHOS protein per individual indicated an overall decrease in mitochondrial protein expression in most PD patients (7 of 9) compared to the controls (Fig. 1B). Furthermore, it highlights the variability in OXPHOS protein expression between individuals from both groups; 2 of 9 PD patients exhibited an astrocytic expression level of all five OXPHOS complexes comparable to most of the controls (PD01, ranged from 0.33 to 1.48; PD08, 0.51–1.83) and the expression of these proteins within SN neurons.13 The remaining 7 PD patients demonstrated varying degrees of decreased OXPHOS protein expression. The lowest expression was observed for ATP5B in PD06 (z median: –2.41), with all other subunits in this case also showing low expression (range: –2.41 to –0.44). Interestingly, 2 of 10 controls showed a widespread decrease of three (Con03) or four (Con10) complexes. Con10 is the oldest control, and we detected a similar pattern of expression changes for these OXPHOS proteins within SN neurons.13

Despite the existence of large heterogeneity in the cohort, significant decreases involving all five OXPHOS complexes were revealed in PD patients compared to the control group (Figure S1A–H; number of astrocytes analyzed: control, n = 477; PD, n = 458). These differences were statistically described using Bayesian estimation,33 which considers the uncertainty caused by varying sample distribution and size per group via computing random sampling. The largest decrease was found for complex IV expression (the mean of difference, 0.572, COXIV; 0.474, MTCO1), whereas the smallest was found for complex III (0.192).

PD Impacts OXPHOS Protein Expression in Astrocytes More Than Age

Mitochondrial dysfunction within SN neurons correlates with advancing age,15 but the impact of age on the expression of OXPHOS proteins in astrocytes is unclear. Linear regression confirmed that, as within neurons, aging impacts the astrocytic expression of some OXPHOS proteins; however, the pattern of these changes differs (Fig. 2A–I). Within astrocytes, the expression of subunits of complexes I and V increases with normal aging (slope = 0.01–0.02, NDUFA13, OSCP, P < 0.05; NDUFB8, ATP5B, P < 0.001), whereas the expression of complex III declines (slope = –0.02, P < 0.001). For complex IV, aging had no significant impact on COXIV expression, but the expression of MTCO1 also increased (slope = 0.01, P < 0.001).

Since PD is an age-related disease, we modeled the impact of age and its interaction with PD on the expression of OXPHOS proteins, using multiple linear regression with interaction modeling.31 A significant effect of this interaction was identified on the expression of complexes I (NDUFA13 and NDUFB8), IV (COXIV and MTCO1), III (UQCRCC2), and V (ATP5B) (P < 0.0001). With the exception of UQCRCC2, the expression of the other five subunits demonstrated declining trends (slope < 0) with age and a PD effect which was reversed compared to the effect of age alone. This indicates that deficiencies in OXPHOS subunit expression should be overwhelmingly attributed to disease rather than aging. Interestingly, this was not true for UQCRCC2 where the inverse was true. The approach also showed that the expression of VDAC1 and SDHA (complex II) was not impacted by age (VDAC1 P = 0.10; SDHA P = 0.09) or its interaction with PD (P = 0.06; 0.29). Importantly, no significance was identified from the regression analysis of parameters associated to tissue quality: postmortem delay, fixation time, and protein expression (figure not shown, P > 0.05).

PD Astrocytes Show Combined OXPHOS Deficiency and at Comparable Levels to Neurons

Given the importance of astrocyte neuronal support, we determined whether OXPHOS deficiency was detected within astrocytes and whether the proportion of deficient astrocytes differed between PD patients and controls (Fig. 3A). Our statistical approach was based on previous neuronal data13 and the hypothesis that a similar proportion of astrocytes would be affected. A significant increase in the percentage of complex I- or complex IV-deficient

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**FIG. 1.** IMC (imaging mass cytometry) profiling of mitochondrial OXPHOS (oxidative phosphorylation) protein expression in the SN (substantia nigra) astrocytes. (A) Images demonstrate IMC analysis of a panel including 12 mitochondrial antibodies within SN astrocytes from a PD (Parkinson’s disease, PD07) patient and an age-matched healthy individual (Con08). The panel targets subunits of all five mitochondrial OXPHOS complexes (complex I-NDUFA13; NDUFB8; complex II-SDHA; complex III-UQCRCC2; complex IV-COXIV; MTCO1; and complex V-ATPSB, OSCP), a mitochondrial mass marker (VDAC1), an astrocyte marker (GFAP [gliarial fibrillary acidic protein], a dopaminergic neuronal marker (TH [tyrosine hydroxylase]), and a nuclear marker (Histone H3). Scale bar, 10 pixels/μm. (B) Original pixel intensities for individual astrocytes were transformed into z scores for normalization, allowing the tested respiratory chain subunits to be normalized relative to mitochondrial mass. Z scores of mitochondrial mass (VDAC1, normalized to the astrocytic area) for individual astrocytes were compared. The relative median z score per individual for each of the nine tested mitochondrial proteins is presented as a heatmap (number of cases analyzed: PD, n = 9; control, n = 10. Number of astrocytes analyzed: control, n = 477; PD, n = 458). Individual case was listed by advancing age (oldest: CON10 and PD09).
astrocytes was found in PD patients compared to controls (Mann-Whitney *P* < 0.05). Complex I deficiency, commonly reported within PD SN neurons, was detected at a comparable level to deficiencies in complex IV (particularly for COXIV). Over 25% of astrocytes showed decreases in both a complex I subunit and the nuclear-encoded complex IV subunit, COXIV in 6 of 9 PD patients (4 of 9 patients for MTCO1). This approach revealed little deficiency within the control astrocytes (median and IQR: NDUFA13, 0 and 4.95; NDUFB8, 0 and 17.11; COXIV, 2.50 and 6.47; MTCO1, 1.81 and 3.32). Further regression analysis on all test cases (PD, n = 9; control, n = 10) confirmed that the percentage of deficient astrocytes for individual OXPHOS complexes (6 of 8 tested subunits) was positively correlated to those identified in the neuronal population in the PD patients (n = 9; Fig. 3B). These subunits are mainly from the core complexes (I, IV, and V), with the strongest correlation identified for both complex V subunits (ATP5B, adjusted *r*² = 0.80, *P* < 0.001; OSCP, adjusted *r*² = 0.46, *P* = 0.03), though no significant trend was found in the control. In contrast to SN neurons, complex III...
FIG. 3. Legend on next page.
deficiencies were rarely detected in astrocytes irrespective of disease status (Fig. 3A). This is also consistent with the observation that the average percentage of complex III–deficient astrocytes is significantly lower than for neurons in PD; however, such a difference was not observed for other complexes (Figure S2).

The number of complexes showing deficiency within individual astrocytes was calculated (Fig. 3C) to evaluate OXPHOS dysfunction severity at a single-cell level, difficult to achieve by immunofluorescence. In 7 of 10 controls, more than 80% astrocytes showed no OXPHOS defect (median, 89.99%), whereas the percentage of PD astrocytes falling into this category was much lower (median, 35%), with the exception of the 2 previously mentioned PD patients (PD01 and PD08) which showed 100% normal astrocytes. In contrast, astrocytes in 7 of 9 patients cases demonstrated severe OXPHOS deficiency involving all five complexes (median, 4.17%). We identified significantly more deficient astrocytes in the PD group, which showed combined deficiencies of two (Mann-Whitney $P = 0.02$) and four ($P = 0.03$) complexes compared to the controls. Overall, these data provide evidence of combined loss of OXPHOS complexes in PD astrocytes, revealing the severity and complexity of OXPHOS defects.

**Lack of Mitochondrial Mass Response to OXPHOS Deficiency in PD Astrocytes**

Our recent study of DN identified a compensatory increase in mitochondrial mass in response to the loss of complexes I and IV in PD.\(^{13}\) Thus, we investigated whether such changes were also detected in astrocytes and particularly in those with OXPHOS deficiency. We focused on three complexes due to their critical roles in the maintenance of electron transport (complexes I and IV) and ATP synthesis (complex V) and because they were significantly affected in PD astrocytes in this study. To account for the impact of variation between individuals on the comparison of group expression levels, linear mixed effects modeling with individual sample size was considered (Fig. 4A, $P = 0.14$).

Intriguingly, significant decreases in mitochondrial mass were detected in PD astrocytes deficient in any of the three tested complexes compared to the normal ones (Fig. 4B–D, $P < 0.001$), coefficient ± standard deviation: $\text{NDUFA13}, -0.44 \pm 0.10$; $\text{MTCO1}, -0.80 \pm 0.10$; $\text{ATP5B}, -0.58 \pm 0.11$). Furthermore, in PD such decreases in mitochondrial mass were observed in those astrocytes deficient in all three complexes compared to those that showed a single deficiency (Fig. 4E, $-0.11 \pm 0.17$, $P < 0.001$) or were not deficient ($-0.71 \pm 0.14$, $P < 0.001$). Interestingly, we found the converse to be true in control astrocytes that were deficient in subunits of these three complexes, with an increase in mitochondrial mass detected in these astrocytes compared to those with a single deficiency ($0.58 \pm 0.33$, $P < 0.001$) or classed as normal ($0.30 \pm 0.21$, $P < 0.001$). This may suggest the occurrence of a glial response to OXPHOS defects in SN astrocytes with normal aging; however, this responsive increase in mitochondrial mass in deficient astrocytes does not seem to occur in PD.

In addition, we found that long disease duration and age of onset could have a combined, aggravating effect on the decline of mitochondrial mass within PD astrocytes (Fig. 4F, $P < 0.001$).

**Mitochondrial Mass Changes in Neuronal and Neuropil Astrocytes**

Given the importance of astrocytic support for DN and the potential contribution of mitochondrial dysfunction to their selective vulnerability,\(^{34}\) we compared astrocytes that were in close proximity to neurons to those within the neuropil. Neuronal astrocytes were those that had a large amount of their area that appeared to be in contact with a TH-positive neuronal cell body, whereas those described as neuropil were found close to blood vessels or in a region with no dopaminergic cell bodies and little TH immunoreactivity (identifying dopaminergic axons or dendrites) (Fig. 4G). The number of astrocytes within the two categories for each individual is summarized in Table S2.

Morphological flexibility and the functional status of brain astrocytes are closely associated. Here, we found that SN neuropil astrocytes were significantly larger compared to the neuronal astrocytes in both PD and

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**FIG. 3.** Comprehensive description of OXPHOS (oxidative phosphorylation) protein deficiency in astrocytes. (A) The percentage of SN (substantia nigra) astrocytes showing a statistically deficient level of protein expression (“deficient” = below 10% of the prediction interval for the control astrocytes) for each individual case was grouped for comparison (Mann-Whitney $P < 0.05$). (B) Regression analysis between these astrocytes and the corresponding neuronal population from the same individual (9 PD patients, 10 controls) was performed (simple linear regression, adjusted $R^2$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). Dots represent individual case. (C) The number of deficient complexes within individual astrocytes from each individual was also compared between the two groups (Mann-Whitney $P < 0.05$). The expression level of each mitochondrial complex protein was transformed into z scores to normalize to mitochondrial mass (VDAC1/Porin). Each dot represents one individual; bars show the median and 75% percentile for each group.
FIG. 4. Legend on next page.
control groups, though this difference appears larger within the disease group (Fig. 4H, $P < 0.001$; PD, 23.42 Hz ± 6.65 vs. control, 3.26 Hz ± 6.01). Meanwhile, neuropil astrocytes exhibited a significantly larger area in PD patients than in the controls (31.71 Hz ± 15.01, $P = 0.05$), with no significant difference detected for neuronal astrocytes ($P = 0.55$).

For mitochondrial mass in general (normalized to area), both PD and control astrocytes showed no significant changes between the two astrocytic populations for the expression of VDAC1 (Fig. 4I). Interestingly, there are significant increases in the expression of complexes IV and V in the control neuronal astrocytes when compared to those in the neuropil (−0.13 Hz ± 0.06, $P = 0.03$; −0.10 Hz ± 0.05, $P = 0.04$), whereas such differences were not detected in PD (data not shown). For complex IV–deficient astrocytes, neuronal astrocytes showed higher mitochondrial mass compared to the neuropil astrocytes in individuals with PD (linear mixed model, 0.40 Hz ± 0.16, $P = 0.02$), whereas an opposing trend (not significant) was observed between the two categories of astrocytes in the control group (Fig. 4J, −0.31 Hz ± 0.4, $P = 0.45$). Moreover, a lower level of mitochondrial mass was found in the PD neuropil astrocytes than in controls (Fig. 4J, −2.53 Hz ± 0.85, $P = 0.02$). The same pattern of changes was shown within the complex I- and V-deficient astrocytes; however with the impact of individual variants, no statistical significance was identified (data not shown). Despite a small sample size, this analysis indicates that mitochondrial mass in astrocytes is altered in PD and there are differences between astrocyte mitochondrial populations depending on their location.

**Discussion**

Though mitochondrial dysfunction within neurons has been extensively studied in PD, the presence and consequence of OXPHOS dysfunction within astrocytes are yet to be established. Here we provide the first evidence for OXPHOS deficiency within SN astrocytes in individuals with PD involving all complexes (except complex III). Furthermore, we detected changes in mitochondrial mass within PD astrocytes, which may impact the function of these important glia.

**Reduced Expression of OXPHOS Proteins within PD Astrocytes**

Although not previously described in detail in human tissue, mitochondrial respiratory deficiencies have been shown to impair astrocyte reactivity and local neuronal survival in a mouse model with astrocytic knockout of mitochondrial transcription factor, TFAM. Unlike neurons, astrocytes are not considered particularly reliant on OXPHOS, predominantly using glycolysis for ATP production; astrocytes, however, generate lactate to support neuronal mitochondrial respiration in response to neuronal activity. Mitochondrial dysfunction within astrocytes impacts both glutamate and calcium homeostasis and may also lead to the release of mtDNA into their cytoplasm, triggering the initiation of the inflammatory STING cascade. Thus, it is clear that mitochondrial dysfunction impacts astrocyte function and responsiveness, potentially leading to further inflammation that may exacerbate neurodegeneration. However, until now the presence of such dysfunction within astrocytes in PD is unknown. Here we confirm that OXPHOS deficiency is present within SN astrocytes in PD, affecting all complexes with the exception of complex III. Similar to the mitochondrial changes detected within SN neurons, deficiencies in complexes I and IV are the most prevalent. Because, one of the primary functions of astrocytes is to support the function and survival of neurons, OXPHOS deficiency within these cells could contribute to the degeneration of DN

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**FIG. 4.** Mitochondrial mass in OXPHOS (oxidative phosphorylation)-deficient PD astrocytes. (A–E) Using linear mixed modelling and individual case as a random effect, z scores of mitochondrial mass (VDAC1) for individual astrocytes were compared (A) between PD (number of astrocytes, n = 458) and control groups (n = 477), (B) between complex I-deficient (PD, n = 72/6 cases; control, n = 28/5 cases) and normal astrocytes (PD, n = 176/6 cases; control, n = 242/5 cases), (C) between complex IV-deficient (PD, n = 80/9 cases; control, n = 32/4 cases) and normal astrocytes (PD, n = 282/9 cases; control, n = 204/4 cases), (D) alongside complex V-deficient (PD, n = 94/5 cases; control, n = 23/4 case) and normal astrocytes (PD, n = 143/5 cases; control, n = 22/4 case) (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). (E) Such comparison was also performed between astrocytes, with the three complexes showing deficiency, a single deficiency, and normal (linear mixed model, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Cases without astrocytes showing no deficiency in either complex I, IV, or V were excluded for the analysis. Boxes show the median, 75% and 25% percentiles; triangles represent the mean. (F) Multiple linear regression with interaction modeled the interfering effects of the age of onset and disease duration on mitochondrial mass within PD astrocytes. Lines represent the fitted regression line (gray line, the age of onset only; yellow line, interacted effects of the age of onset and the disease duration; $R^2 = 1.08$, RMSE = 0.35); each dot represents an individual astrocyte. (G) Two categories of SN (substantia nigra) astrocytes (GFAP [glial fibrillary acidic protein], red) were defined based on their proximity to dopaminergic neuronal cell bodies as identified by positive tyrosine hydroxylase (TH [tyrosine hydroxylase], green) and intracellular neuromelanin (intercalator, navy) staining. Neuronal astrocytes (left, white outline) were defined based on their close proximity to dopaminergic neurons (left, yellow outline), whereas neuropil astrocytes were those that were located at a distance from the neuronal population (right). Scale bar, 10 pixels/μm. (H) Area of individual outlined astrocytes were analysed for the two astrocytic populations and compared between the groups. (I) Such analysis was also performed for mitochondrial mass per astrocytic area, VDAC1 (PD: neuronal, n = 240; neuropil, n = 218; Control: neuronal, n = 258; neuropil, n = 218). (J) For those astrocytes showing deficiency in complex IV, changes of mitochondrial mass was also shown between the two astrocytic populations and between PD and controls (PD: neuronal, n = 50; neuropil, n = 30; case number, n = 9; Control: neuronal, n = 16; neuropil, n = 16; case number, n = 4). Boxes show the median, 75% and 25% percentiles; triangles represent the mean (Linear mixed model with individual case as a random effect; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)
in PD. Fiebig et al recently showed that respiratory deficiency within astrocytes actually increased neuronal loss associated with ischemic lesions, suggesting that mitochondrial dysfunction had led to an impairment of their neuroprotective abilities. Interestingly without compromising the survival of the astrocytes themselves. Therefore, the increase in OXPHOS deficiency in PD astrocytes may compromise their ability to support dopaminergic neuronal survival, contributing to the excessive loss of neurons in PD.

**Alterations in Mitochondrial Mass and Impact in PD**

The mitochondrial population within astrocytes is considered comparable to that within neurons, though astrocytes are considered more metabolically dynamic than neurons. It is considered that they are able to alter and adapt their metabolism in response to both injury and pathologic states. Mitochondrial dysfunction within neurons contributes to the pathogenesis of PD, and importantly some studies have suggested that astrocytes may play an active role in the maintenance of neuronal mitochondrial populations. By removing dysfunctional mitochondria for degradation, astrocytes may act to protect neurons against mitochondrial dysfunction; alternatively, they may donate functional mitochondria to neurons in response to stress or injury. By considering the differential sample size of each individual, we did not identify significant changes in mitochondrial mass between PD and control astrocytes when considering all analyzed astrocytes. However, the mitochondrial mass of astrocytes with and without deficiency of complexes I, IV, and V differed between controls and PD patients. In controls, mitochondrial mass was found to be higher in astrocytes, which were characterized as being deficient in complexes I, IV, and V compared to normal astrocytes; however, this was not the case in PD. In PD patients, astrocytes classed as deficient showed a significantly decreased mitochondrial mass compared to normal astrocytes, with further differences identified when astrocytes in close proximity to neurons were compared to those at a distance. The interpretation of this finding might suggest that mitochondria are removed from neurons by astrocytes for degradation, which may be supported by the trend for increased mass in deficient astrocytes in controls. This might be an attempt to mitigate the OXPHOS defects, which would increase the mass of neighboring astrocytes. A decrease in mass in PD may suggest impairment of this transfer. Therefore, perhaps astrocytic transfer and degradation of neuronal mitochondria within astrocytes is impaired, creating a failure of support for neurons with OXPHOS deficiency that may lead to their death.

In addition, the lack of OXPHOS dysfunction within control astrocytes may indicate an upregulation of mitochondrial mass to donate functional mitochondria to neurons or to increase the capacity to provide lactate to neurons with mitochondrial dysfunction. Under normal circumstances this would support neuronal function and survival but may exacerbate mitochondrial defects if already present within the neuron. The possibility would again be that this support of neuronal mitochondrial populations cannot occur in PD and may actually be detrimental; thus, the neurons are more likely to be overwhelmed by their mitochondrial defects and degenerate. Finally, the detection of OXPHOS deficiencies in astrocytes may further support the notion that dysfunctional mitochondria are transported to astrocytes for degradation. Astrocytes are proliferative, whereas neurons are not, which contributes to the acquisition and expansion of mitochondrial dysfunction within them. Thus, astrocytes supporting neurons may be younger than the cells they support; thus, the fact that we see a strong correlation between the percentage of deficiency between the two cell types in PD may suggest a transfer of defective mitochondria to astrocytes. However, this could negatively impact the function of the astrocytes, leading to a loss in their support of neurons or exacerbation of inflammatory processes, including recruitment of microglia that may actually progress neurodegeneration. For the understanding of any of these scenarios, further work is required to model the dynamics of these processes and to determine their contribution to PD pathogenesis.

**Conclusion**

Here we provide the first in-depth study examining the expression of essential OXPHOS proteins in astrocytes in PD. The capacity of astrocytes to support neurons and their function is essential for neuronal survival. Despite not being reliant on OXPHOS for cellular functions, OXPHOS defects are common in astrocytes in PD, alongside alterations in mitochondrial mass. These alterations suggest that astrocytic support of DNs is impaired in PD and may contribute to the neuronal loss present in affected individuals.

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Data Availability

Raw datasets for this study (for all figures and supplementary materials) are available in the ‘Figshare’ repository (10.25405/data.ncl.16635694). Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.