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Mitochondrial Abnormalities in the Putamen in Parkinson's Disease Dyskinesia

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Abstract

Prolonged treatment of Parkinson's disease with levodopa leads to disabling side effects collectively referred to as 'dyskinesias'. We hypothesized that bioenergetic function in the putamen might play a crucial role in the development of dyskinesias. To test this hypothesis we used post-mortem samples of the human putamen and applied real time-PCR approaches and gene expression microarrays. We found that mitochondrial DNA levels are decreased in patients who have developed dyskinesias, and mitochondrial DNA damage is concomitantly increased. These pathologies were not observed in Parkinson's disease subjects without signs of dyskinesias. The group of nuclear mRNA transcripts coding for the proteins of the mitochondrial electron transfer chain was decreased in patients with dyskinesias to a larger extent than in patients who had not developed dyskinesias. To examine if dopamine fluctuations affect mitochondrial DNA levels in dopaminergic neurons, rat striatal neurons in culture were repeatedly exposed to levodopa, dopamine or their metabolites. Mitochondrial DNA levels were reduced after treatment with dopamine, but not after treatment with dopamine metabolites. Levodopa led to an increase in mitochondrial DNA levels. We conclude that mitochondrial susceptibility in the putamen plays a role in the development of dyskinesias.

Keywords

Parkinson's disease; dyskinesia; putamen; mitochondria; respiratory chain

Introduction

Parkinson's disease (PD) is the consequence of a degeneration of dopaminergic neurons in the substantia nigra (SN) pars compacta. Although some cases of PD are of genetic origin [34], the majority of cases are of unknown etiology. Mechanisms common to the genetic and epidemiological factors in PD include mitochondrial pathology [33,42], oxidative stress [24, 41,44] and defective protein recycling through the proteasome system [32].

PD is treated with the DA precursor levodopa (L-dopa) [5], combined with the peripheral decarboxylase inhibitor carbidopa. This treatment is successful in the early stages of the disease. As PD progresses, patients develop abnormal involuntary movements such as chorea,

dystonia and myoclonus (dyskinesias), which are painful and disabling side effects of L-dopa treatment [23].

Research focused initially on the causes for neuronal degeneration. However, since 70–80% of dopaminergic neurons are lost before the first symptoms of PD emerge [4], the disease manifests itself after most of the pathological damage has happened. While knowledge of the factors that lead to the degeneration of DA neurons is important, such knowledge might not be sufficient to prevent the disease in the many cases of unknown etiology. Because clinical studies have not discovered viable alternatives to L-dopa treatment, the research focus is shifting toward understanding how L-dopa causes dyskinesias [9]. Preventive measures can then be taken simultaneously with L-dopa therapy to avoid the development of dyskinesias.

An important factor in the degeneration of DA neurons in the SN is the impaired mitochondrial function and oxidative stress caused by DA and its metabolites [8,12,25,31]. Based on the knowledge that DA is a source of oxidative stress [21], and that DA D₁ receptors facilitate glutamate/NMDA receptor activity in the striatum [15], we hypothesized that dyskinesias might be the consequence of cellular stress and reduced bioenergetic function due to heightened fluctuations of DA levels in the putamen caused by the combination of PD and L-dopa treatment.

The putamen is one of the main target areas of DA axons, and the part of the striatum with the largest DA deficiency in PD [4]. DA deficiency leads to DA receptor supersensitivity, and D₁ receptor supersensitivity in the striatum has been linked to the development of dyskinesias [9,18]. In supersensitive D₁ receptor-expressing neurons, temporary spikes in DA after L-dopa treatment could disproportionately facilitate NMDA receptor activity [15] and cause temporary increases in ion flux. Elevated glutamate receptor activity can lead to mitochondrial Ca²⁺ loading, decrease in mitochondrial viability and bioenergetic function, and increased cellular stress [7].

Mitochondria in the brain of PD patients are under significant stress. In the SN, PD patients have increases in a frequently observed mtDNA deletion [2]. This ‘common’ deletion is associated with cytochrome C oxidase deficiency and oxidative stress [2,28,35]. The deletion is also prevalent in the putamen of the general population, suggesting an overall vulnerability of this brain area [13].

Here we examined mitochondrial DNA (mtDNA) and RNA properties of PD patients who had developed dyskinesias and compared them to patients who had a stable motor response, and to normal control subjects who had no neuropathological abnormalities. The impact of L-dopa, DA and their metabolites on striatal mtDNA levels was furthermore examined in primary rat striatal cultures.

Materials and methods

Sample collection

All samples were collected at the Harvard Brain Tissue Resource Center at McLean Hospital (HBTRC; <http://www.brainbank.mclean.org>). A round sample corer of 8 mm diameter was used to obtain samples of approximately 2–3 mm thickness from the mid-putamen from a coronal slice of the brain at the level of the optic chiasm. Clinical diagnosis was verified through a comprehensive neuropathological examination at the HBTRC. All medical records were carefully examined for any symptoms attributable to L-dopa-induced dyskinesias, under observation of all HIPAA and IRB guidelines and with the help of a questionnaire developed by the investigators. Medical records were scanned for terms such as ‘dyskinesia’, ‘chorea’, ‘wearing off’, ‘on-off periods’ and prescription of Comtan (entacapone) or tolcapone (Tasmar),

and recorded on a questionnaire together with information on medications, signs of PD, scales and tests administered, L-dopa prescription records, neuropathological findings, signs of dementia and cause of death. Exclusion criteria included incomplete medical records, exposure to environmental toxins and requirement of respiratory devices before death. Because the number of female samples with complete medical records was small and uneven across the groups, analyses were restricted to males (PMI; table S1). Investigators were blinded to the diagnosis.

DNA and RNA extraction

DNA was extracted using the QIAGEN DNeasy kit; for RNA extraction, the RNagent kit was used (Promega Corporation, Madison, WI). Tissue was suspended in denaturing solution from the RNagent kit and homogenized with a PowerGen 700 homogenizer. All samples were examined in an analytical gel and with the bioanalyzer 2100 (Agilent, Santa Clara, CA). Samples with RIN numbers above '6' were included in the initial workup (average 7.2 ± 1.1 SD). Samples not meeting the quality criteria outlined below were removed.

qPCR reactions

Determination of mtDNA levels—qPCR analysis was carried out with 50 ng of DNA and six mitochondrial DNA primer pairs used in individual reactions in duplicates for human samples (Mt1.1 to 7.1, table S2A), or four mitochondrial DNA primer pairs used in individual reactions in duplicates for rat samples (table S2B). SYBR green was used for detection of double-stranded DNA, and a meltcurve analysis from 55°C to 90°C was performed after the last PCR cycle to examine melt-temperature and melt-peak. Each plate had its own standard curve of five 1:5 dilutions of a sample consisting of equal aliquots of all samples. Data were retrieved from the standard curve. The highest and third dilutions of the standard curve, plus randomly selected samples were chosen from each plate to be examined on a 5% vertical acrylamide:bisacrylamide (37.7:1; Protogel, National Diagnostics, Atlanta, GA) gel for verification of product size and specificity. All data are normalized to an average of nuclear GAPDH DNA and total DNA input (no difference was observed between them), both averaged to '1' for equal weight. Data of rat mtDNA primer pairs were averaged and normalized to nuclear β -actin DNA and alpha1A tubulin DNA, averaged (table S2B). Because the number of DNA copies per cell of any given gene is constant, the target choice has no influence on the levels measured.

Determination of RNA levels—One μ g of total RNA was used for complementary DNA (cDNA) synthesis with the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). The iQ SYBR Green supermix (Bio-Rad) was used for the PCR reaction. Six normalization controls were used and averaged (table S2C).

Primer design—Primer-Blast (based on Primer3) was used for the primer design [39]. All primers were purchased from Integrated DNA Technologies (Coralville, IA).

MtDNA deletion analysis

The percentage of 'common' mtDNA deletion was determined with a multiplex PCR method followed by quantification of PCR products with Bioanalyzer technology (Agilent Technologies, Santa Clara, CA). PCR products were obtained from the 'common' deletion area and a stable area of the mitochondrial plasmid, and the percentage of non-deletion was calculated based on molar concentration of each PCR product (primer pairs table S2A, see also [28]).

Gene expression microarrays

Affymetrix Human U133A2.0 Arrays (Affymetrix, Santa Clara, CA) were used for gene expression analysis. Four μg of total RNA was used for complementary DNA (cDNA) synthesis using the SuperScript double-stranded cDNA synthesis kit (Invitrogen Corp., Carlsbad, CA) and an oligo (dT) primer with T7 promoter sequence. Biotinylation was carried out with the Gene Chip Expression 3' Amplification kit for IVT (Affymetrix,). Hybridization to the array and washing and staining were performed according to company protocol. Samples from individual subjects were hybridized to individual arrays. Only samples that reached commonly accepted quality control criteria defined by Affymetrix, dChip [30] and RMAExpress [6], were used in the analysis.

Programs used for data collection included GeneChip Operating Software (GCOS, Expression Console) (Affymetrix) for scanning and to obtain quality control data, RMAExpress [6] for quantile normalization and background correction to compute expression levels for all probe sets, DNA-Chip Analyzer [30] for clustering, and David 6.7 [14] to group regulated genes into functional annotations provided by a number of databases (see <http://david.abcc.ncifcrf.gov/>). Expression values were log2-transformed to improve normal distribution in all analyses and p-values for significant terms were computed using false-discovery correction [3].

Primary rat striatal cultures and HPLC analysis

Striata were dissected from 18-day old Sprague-Dawley rat fetuses (Charles River Laboratories, Raleigh, NC) according to previously published protocols, and cells were seeded in 12-well plates at 1.2×10^6 cells per well [36]. Cultures were treated with 5 μM of either dopamine (DA), L-3,4-dihydroxyphenylalanine (L-dopa), or the DA metabolites 3-methoxytyramine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) on days in vitro (DIV) 3, 6, and 9, and harvested on DIV 10, 18 hours after the last addition of drugs. Cultures were monitored daily for loss of cells or changes in cellular morphology such as retraction of cellular processes or cell body granulation. The average amount of DNA extracted from each group on the day of the experiment was 7 μg per well, with no difference between groups. Since human cells contain 6 pg of DNA, the yield corresponds to 1.2×10^6 cells in each group, the number of cells initially seeded.

On DIV 10, 18 hours after the last treatment, 1 ml of culture medium was aspirated and subjected to HPLC analysis. The Neurochemistry Core at Vanderbilt University carried out the analysis.

Statistical Analysis

ANOVAs and ANCOVAs with age and PMI as covariates were applied in most analyses, followed by post-hoc t-tests. The JMP computer program (SAS Institute, Cary, NC) was used for all analyses.

Fisher's exact test was applied to determine if the number of significant probe sets of the mitochondrial respiratory chain complex exceeded expectations. The probe sets had to be present in at least 80% of samples per group and a p-value of ≤ 0.05 .

Results

Patients were matched for age, gender (all males) and PMI. Differences between dyskinetic-PD samples and non-dyskinetic-PD samples remained for duration of disease, total amount of L-dopa prescribed during the entire treatment period ('total lifetime L-dopa'), and average amount of L-dopa prescribed per year (table S1), due to the fact that dyskinesias develop as the disease progresses (figure 1a). With increased disease duration the dosage of L-dopa was

generally increased, but at equivalent disease duration patients in both groups received equivalent L-dopa prescriptions (figure 1b; $F_{1,29}=20.3$, $p<0.0001$, $r^2=0.41$; no significant difference for diagnosis). Total lifetime L-dopa was correlated with disease duration (figure 1c; $F_{1,29}=35.4$, $p=0.0001$, $r^2=0.55$; no significant difference for diagnosis), but the slope between both groups was not significantly different. Thus, subgroups of patients could be matched for disease duration, average amount of L-dopa p.a, and total lifetime L-dopa exposure.

MtDNA levels were significantly different between diagnostic groups in the putamen (ANCOVA for 'diagnosis' with the covariates 'age' and 'PMI', $F_{4,35}[\text{diagnosis}]=10.8$; $p<0.001$; figure 2a), but not different in the cerebellum (figure 2b). Post-hoc analysis of the putamen samples showed a 50% reduction in mtDNA levels of dyskinetic-PD subjects compared to controls ($F_{3,22}[\text{diagnosis}]=14.4$, $p<0.001$; ANCOVA corrected for 'age' and 'PMI'). That difference was still present in a subgroup of samples matched for disease duration, age, average L-dopa per year and PMI (figure 2c). Dyskinetic-PD patients had lower levels of mtDNA earlier in the disease process (figure 2d), as indicated by a significant negative correlation between mtDNA levels and disease duration in these samples ($F_{1,8}=23.6$, $p<0.001$, $r^2=0.747$). No significant correlation was observed between mtDNA levels and disease duration in non-dyskinetic-PD samples ($F_{1,12}=2.6$, $p<0.130$, $r^2=0.18$).

Average annual prescription levels of L-dopa had a negative effect on mtDNA levels as well, but did not influence the significant difference between dyskinetic-PD and non-dyskinetic-PD samples ($F_{4,19}[\text{diagnosis}]=12.9$, $p<0.002$; ANCOVA corrected for 'age', 'PMI' and 'average L-dopa p.a.'). A significant negative correlation between mtDNA levels and average L-dopa per year was observed in dyskinetic-PD samples only ($F_{1,8}=12.3$, $p<0.008$, $r^2=0.61$), but not in non-dyskinetic-PD samples ($F_{1,11}=1.7$, $p<0.213$, $r^2=0.13$; figure 2e). These results were comparable to results obtained with total lifetime L-dopa exposure ($F_{1,8}=6.9$, $p<0.03$, $r^2=0.46$ for dyskinetic-PD, n.s. for non-dyskinetic-PD).

We further analyzed mtDNA for a previously described "common deletion" which is a marker for mitochondrial damage secondary to oxidative stress [2,28,35]. MtDNA deletions showed a significant group difference (ANCOVA for 'diagnosis' with the covariates 'age' and 'PMI', $F_{4,40}[\text{diagnosis}]=5.8$; $p<0.006$), and post-hoc analysis confirmed that the dyskinetic-PD group differed from the control group ($F_{3,27}[\text{diagnosis}]=10.0$, $p<0.004$; ANCOVA with the covariates 'age' and 'PMI'; figure 3a). MtDNA deletions were neither correlated with L-dopa levels, nor with disease duration in the overall sample or in the diagnostic subgroups. Because levels of PCR product in the deleted region were normalized to PCR product in the stable region within each sample, these data demonstrate that the dyskinetic-PD putamen had a higher percentage of deletions. We also observed a correlation between mtDNA levels and mtDNA deletions ($F_{1,37}=7.5$, $p<0.001$, $r^2=0.16$; figure 3b) in the PD samples ($F_{1,22}=4.4$, $p<0.049$, $r^2=0.17$; figure 3b) which was not observed in the control samples (figure 3b).

A subset of samples matched for age, gender, PMI, average amounts of L-dopa prescribed per year and disease duration (figure 4a) was subjected to gene expression microarray analysis. The NIH David Tool demonstrated that mitochondria-related mRNAs were the most significantly overrepresented group of downregulated genes in dyskinetic-PD samples compared to control samples. Among the mitochondria-specific GO terms were 'mitochondrial membrane part', $p=2.3E-11$; 'mitochondrion', $p=4.5E-11$; 'mitochondrial inner membrane', $p=3.7E-9$ and 'oxidative phosphorylation', $p=4.1E-8$. All p-values were corrected according to [3]. Fisher's exact test showed that the percentage of downregulated mETTs was significantly higher than expected, whereas the percentage of upregulated mETTs was significantly lower than expected (figure 4b; table S3).

The most prominent downregulated transcripts in the comparison of non-dyskinetic-PD samples to control samples were also related to mitochondrial function (GO terms 'mitochondrial membrane part', $p=2.3E-8$; 'mitochondrion', $p=4.7E-8$; 'mitochondrial inner membrane', $p=1.6E-8$; 'oxidative phosphorylation', $p=9.1E-8$; all p -values after Benjamini-Hochberg correction [3]). In non-dyskinetic-PD samples the percentage of downregulated mETTs was significantly higher than expected, whereas the percentage of upregulated mETTs was significantly lower than expected (figure 4b; table S3).

A comparison between the dyskinetic-PD group and non-dyskinetic-PD group showed that the downregulation of mitochondrial transcripts was more pronounced in the dyskinetic-PD group. These data yielded significant EASE scores (a modified Fisher's Exact P-Value, [14,22]). The affected GO groups were 'mitochondrion' ($p=0.00024$), 'mitochondrial part' ($p=0.00089$), 'mitochondrial membrane' ($p=0.0061$) and 'mitochondrial inner membrane' ($p=0.0075$; figure 4b; table S3).

Because mETTs are of special interest in PD, individual gene array data for the electron transport chain are shown in table S3. Primer pairs for seven genes were used for qPCR verification, confirming the reduction of mETTs in the dyskinetic-PD group (figure 4c).

To examine if DA or its metabolites can cause a downregulation of mtDNA levels in neurons of the putamen, we carried out experiments in rat primary striatal cultures. DA led to a 25% downregulation of mtDNA levels after three treatments over 10 days (figure 5a). None of the DA metabolites had such an effect. L-dopa caused an increase in mtDNA levels (overall ANOVA for all treatments $F_{5,246}=12.7$, $p<0.0001$). HPLC analysis of the media was carried out to control for metabolism of the different substrates (figure 5c–f). DA and DOPAC were undetectable 18 hours after addition (figure 5c, 5e) and fully metabolized to their products.

Cell death is unlikely responsible for the observed downregulation of mtDNA levels in the cultures, as the amount of total DNA yielded from each experimental group corresponded to the number of cells initially seeded. We also tested for DNA fragmentation by amplifying two different DNA areas in Q-PCR reactions, of 220 and 175 bp length (Table S2B). The amount of amplified DNA product was similar for all groups. Since DNA fragmentation would preclude amplification, the results rule out DNA fragmentation in DA-treated cells. In preliminary experiments ($n=6$ samples per condition), DA and L-dopa at 500 nM concentration had no effect, suggesting that the intensity of spikes in DA levels factors into DA toxicity.

Discussion

In line with previous observations, the rates of dyskinesias were positively correlated with PD duration, and with prescription levels- and treatment duration of L-dopa. The incidence of dyskinesias is increased with prolonged treatment, such that patients treated with L-dopa for 4–6 years have a 40% likelihood to experience motor fluctuations and dyskinesias, and a 90% likelihood by 10 years of treatment [1]. As the response to L-dopa diminishes with the progression of the disease, dosage is usually increased [40], a known risk factor for the development of dyskinesias [16]. Thus, patients who have the disease longer have the combined burden of longer treatment duration and higher L-dopa dosage, which collectively increase the risk factors for dyskinesias.

MtDNA levels were reduced and mtDNA mutations were increased in the putamen after prolonged L-dopa treatment. These alterations were not observed in the cerebellum, which has also been shown to be more resistant to mtDNA mutations during aging [13]. Our data suggest that L-dopa treatment might have a negative effect on mtDNA levels in the putamen. Patients who develop dyskinesia earlier might do so because of increased mitochondrial susceptibility in the putamen, but not the cerebellum. In support of this notion, mtDNA levels in dyskinetic-

PD patients were negatively correlated with average L-dopa exposure per year, as well as with overall L-dopa exposure, while non-dyskinetic-PD samples showed no correlation. Loss of mtDNA copy number is associated with aging [29] and might be linked to nuclear or mtDNA mutations [10]. Thus, it is not surprising that the reduction in mtDNA levels was accompanied by an increase in mtDNA deletions, though this correlation was only observed in the more vulnerable dyskinetic-PD samples. mtDNA deletions are a sign of mitochondrial pathology and oxidative stress [2,28,35], and like loss of mtDNA copy number, damage to mtDNA in the brain is correlated with aging and neurodegenerative disorders [43].

Overall, the data suggest an inherent vulnerability of the mitochondria of the putamen in some PD patients, rendering them more susceptible to develop dyskinesias. Other PD patients seem to have a higher resilience to mitochondrial damage which could prolong the time to manifestation of dyskinesias.

The post-mortem data could not disambiguate between abnormal mitochondria in the cell bodies of the putamen versus abnormal mitochondria in afferent axon terminals, such as the remaining DA axons from the SN. The reduction in nuclear mRNA levels of mETTs in dyskinetic-PD samples provided some evidence for mitochondrial abnormalities in cell somata of the putamen, since the majority of mRNA is localized to cell bodies rather than axon terminals. Dyskinetic-PD samples as well as non-dyskinetic-PD samples showed a reduction in mETTs, with the effect in dyskinetic-PD samples stronger than in non-dyskinetic-PD samples.

To examine if L-dopa or its metabolites could be responsible for the downregulation of mtDNA levels in striatal neurons, we studied primary cultures of the rat striatum. We found that DA reduced mtDNA levels, indicating that DA has a detrimental effect on medium spiny neurons. In contrast, L-dopa had a protective effect, the mechanism of which is currently unknown. Thus, the L-dopa metabolite DA seems responsible for mitochondrial abnormalities.

HPLC analysis of the tissue culture media showed that DA was readily metabolized, but the metabolites were nontoxic to mtDNA levels. Metabolism of DA was mostly accomplished by catechol-O-methyl transferase (COMT), while metabolism by monoamine oxidase (MAO) was low. Considering that glial cells, the predominant harbingers of MAO in the striatum [27], are largely eliminated from the neuronal cultures [11], and that COMT is located in neurons [26], this shift in metabolism is a reflection of the culture conditions, but should not lead to conclusions about metabolism ratios in intact tissue.

How comparable is the DA concentration applied to the cultures to DA levels *in vivo*? Although *in vivo* baseline levels of DA are in the nanomolar range [19], burst spike-firing increases DA levels from hundreds of μM to mM levels [17,19,20]. Moreover, the majority of D_1 -like receptors in the striatum require μM DA levels for activation [37,38]. Thus, it seems reasonable to assume that striatal neurons are subjected to DA bursts of similar and higher concentrations to the ones used in the primary neuron culture experiment. In a homeostatic system these fluctuations are balanced by attenuating factors. In a partially denervated system, such as primary striatal cultures or the PD putamen, adaptive mechanisms change the sensitivity of receptors and increase vulnerability of neurons.

The reduced level of mtDNA and the increase in mtDNA deletions observed in the putamen in PD and dyskinesia-PD indicate mitochondrial pathology. We do not know if there are less mitochondria in the dyskinetic-PD putamen, or if the number of DNA plasmids per mitochondrion are reduced, but the strong correlation of both markers with dyskinesias suggests that mitochondrial distress in the putamen plays a role in the development of dyskinesia. Any pharmacological approach that can reduce mitochondrial stress and prevent

the bioenergetic decline has the potential to delay disease progression and the development of dyskinesias in patients with PD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Demographic data for PD samples

Disease duration was significantly longer in dyskinetic-PD patients, **a**. L-dopa dosage was increased over time, **b**. Because dyskinetic-PD patients tended to have had PD longer, they received higher annual concentrations of L-dopa, and had had more overall exposure to L-dopa during their lifetime, **b**, **c**. However, at similar disease duration timepoints both diagnostic groups of patients had comparable prescription levels of L-dopa. The original cohort consisted of 15 dyskinetic-PD patients, 16 non-dyskinetic-pD patients, and 32 controls, matched for gender, age and PMI (see also table S1).

Figure 2. Decreased mtDNA levels in the dyskinetic-PD putamen, but not cerebellum

MtDNA levels were reduced in dyskinetic-PD samples in the putamen, **a**, **c**, but not cerebellum, **b**. A subgroup of putamen samples, matched for duration of disease and L-dopa exposure, yielded comparable results, **c**. Insert in panel C shows years of PD diagnosis in the matched groups. Disease duration, **d**, and average L-dopa p.a., **e**, were significantly correlated with mtDNA levels in the dyskinetic-PD group, but not in the non-dyskinetic-PD group. **a**, **b**, **d**, **e**, N=16 control samples, 10 dyskinetic-PD samples, 14 non-dyskinetic-PD samples. **c**, N=12 control samples, 7 dyskinetic-PD samples, 8 non-dyskinetic-PD samples. Post-hoc t-test ** $p \leq 0.01$, * $p \leq 0.05$.

Figure 3. Increased number of mtDNA deletions in the dyskinetic-PD putamen

The relative ratio of PCR product in the putamen amplified from the deleted mtDNA region over PCR product from the stable region is reduced in dyskinetic-PD, **a**. N=20 control samples, 11 dyskinetic-PD samples, 14 non-dyskinetic-PD samples. Increased deletion correlated with decreased mtDNA levels in the PD samples, **b**. Lower levels of mtDNA were correlated with higher levels of deletion in the dyskinetic-PD group, but not in the non-dyskinetic-PD group. N=16 control samples, 10 dyskinetic-PD samples, 13 non-dyskinetic-PD samples. Note: Less product of the deleted area indicates more mtDNA deletion. Post-hoc t-test ** $p < 0.01$.

Figure 4. Decreased mRNA levels of genes coding for the mitochondrial electron transport chain in the dyskinetic-PD putamen

A subgroup of samples were matched for disease duration and L-dopa exposure, **a**. The percentage of probe sets on the Affymetrix gene chip coding for the five complexes of the mitochondrial electron transport chain (mETTs) that were significantly regulated in the various comparisons ($p \leq 0.05$, $\geq 80\%$ 'present' call in each group) was compared to the percentage of *all* probe sets regulated in the individual comparisons, **b**. Positive values signify upregulation, negative values signify downregulation. More probe sets than expected were downregulated, whereas less probe sets than expected were upregulated. $N=15$ control samples, 7 dyskinetic-PD samples, 7 non-dyskinetic-PD samples. Fisher's exact test ** $p \leq 0.01$, *** $p \leq 0.001$.

qPCR analysis of a subset of these genes supports the microarray data, **c**. Gene IDs are shown above each histogram; for gene names see table S3. $N=10$ control samples, 7 dyskinetic-PD samples, 11 non-dyskinetic-PD samples. T-test * $p \leq 0.05$

Figure 5. Effects of DA and its metabolites on striatal neurons in culture

a: DA treatment reduces mtDNA levels in rat primary striatal culture. N= 159 controls, 12 L-dopa, 33 DA, 16 3-MT, 16 DOPAC and 16 HVA. Post-hoc t-test * $p \leq 0.05$, *** $p \leq 0.001$.

b: L-Dopa, DA, their metabolites and metabolizing enzymes. AAAL=aromatic amino acid decarboxylase; COMT= catechol-O-methyl transferase; MAO-B= monoamine oxidase type B.

c–f: HPLC analysis of the medium demonstrates that cultures metabolize DA to 3-MT and HVA, **c**; 3-MT partially to HVA, **d**; DOPAC fully to HVA, **e**; do not metabolize HVA, **f**. 3–24 samples per condition