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Differential mitochondrial morphology in ventral striatal projection neuron subtypes

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Abstract

The two striatal projection neuron subtypes (medium spiny neurons- MSNs), those enriched in dopamine receptor 1 vs 2 (D1-MSNs and D2-MSNs) display dichotomous properties at the level of the transcriptome, projections, morphology, and electrophysiology. Recent work illustrates dichotomous mitochondrial length in NAc MSN subtype dendrites after cocaine self-administration, with a shift toward smaller mitochondria, due to enhanced fission, occurring in D1-MSN dendrites and a shift toward larger mitochondria in D2-MSN dendrites. However, to date there has been no comparison of mitochondrial morphological properties between MSN subtypes. In this study we examine mitochondrial morphology in NAc D1-MSNs vs D2-MSNs. We observe an increase in the frequency of smaller length mitochondria in D2-MSN dendrites relative to D1-MSN dendrites, while D1-MSN dendrites display an increase in larger length mitochondria. The differences in mitochondrial length occur in both NAc core and shell, although to a greater extent in NAc core. Finally, we demonstrate that the mitochondrial fusion molecule, Opa1, is differentially expressed in NAc MSN subtypes, with D1-MSNs displaying higher expression of Opa1 ribosome-associated mRNA. The difference in Opa1 levels may account for the bias toward enhanced smaller mitochondria in D2-MSNs and enhanced larger mitochondria in D1-MSNs. Collectively, our study demonstrates differential mitochondrial size and a potential molecular mediator of these mitochondrial differences in NAc MSN subtypes.

Graphical abstract

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Author Contributions

R.C. M.K.L. designed experiments. R.C. and C.C. performed experiments. R.C., C.C., and M.K.L. wrote the manuscript.

Conflict of Interest Statement

The authors have declared no conflict of interest.

Data Accessibility

Raw data will be shared with the research community upon request.

Nucleus accumbens D1-MSN vs. D2-MSN dendrites display differential mitochondrial lengths, with smaller mitochondria occurring in D2-MSN dendrites. This differential size in mitochondria is potentially mediated through the mitochondrial fusion molecules, Opa1, which is differentially enriched in these two neuron subtypes.

Introduction

The striatal medium spiny neuron subtypes (MSNs; a.k.a.- spiny projection neurons), display distinct and often differential properties at the level of the transcriptome, projections, morphology, and electrophysiology. These neuron subtypes exhibit differential enrichment of transcripts (Gerfen et al., 1990; Heiman et al., 2008; Lobo et al., 2006) including dopamine receptor 1 vs 2 (D1-MSNs vs D2-MSNs). In the dorsal striatum the D1-MSNs project to globus pallidus internal and midbrain regions including substantia nigra, while in ventral striatum (nucleus accumbens-NAc) they project to ventral pallidum, lateral hypothalamus, and midbrain regions including the ventral tegmental area (Gerfen et al., 1990; O'Connor et al., 2015; Smith et al., 2013). In contrast D2-MSNs project primarily to pallidal regions including globus pallidus external (dorsal striatum) and ventral pallidum (NAc), although NAc D2-MSNs also send projection to lateral hypothalamus. Further, studies have demonstrated distinct electrophysiological properties in MSN subtypes that correspond to morphological properties (Ade et al., 2008; Cepeda et al., 2008; Chan et al., 2012; Chuhma et al., 2011; Gertler et al., 2008; Kreitzer & Malenka, 2007; Ma et al., 2013; Pascoli et al., 2014; Willett et al., 2018). These properties are altered in MSN subtypes in diseased states including neurodegeneration, addiction, stress- induced affective behavior, pain, and repetitive behaviors (Chen et al., 2017; Fieblinger et al., 2014; Francis et al., 2015; Francis et al., 2017; Galvan et al., 2012; Graziane et al., 2016; Hearing et al., 2016; Heinsbroek et al., 2017; Kim et al., 2011; LeGates et al., 2018; Lim et al., 2012; Massaly et al., 2019; Ren et al., 2016; Rothwell et al., 2014; Schwartz et al., 2014; Terrier et al., 2016).

These differential properties including their projection circuitry through the brain can often give rise to differential action, motivational, and emotional behavioral outcomes (Creed et al., 2011; Francis et al., 2015; Ferguson et al., 2011; Heinsbroek et al., 2017; Hikida et al., 2010; Kravitz et al., 2010; Kravitz et al., 2012; LeBlanc et al., 2018; LeGates et al., 2018; Lobo et al., 2010; Massaly et al., 2019; Rothwell et al., 2014). Additionally, in some of these pathological states mitochondrial properties are disrupted in MSNs (Chandra, Engeln, et al., 2017; Hollis et al., 2015; Larrieu et al., 2017). Mitochondria play key roles in neurons, and are involved in cell physiology, buffering of calcium levels, ROS maintenance, and regulation of neuronal plasticity (Li et al., 2004; Rueda et al., 2014; Vos, Lauwers, & Verstreken, 2010). Further, mitochondria can undergo fission, the division of mitochondria, or fusion, the fusion of two or more mitochondria together through their molecular machinery. Outer membrane protein Dynamin-1-like protein (Drp1) and mitochondrial fission 1 molecules (Fis1) are involved in mitochondrial fission; while inner mitochondrial membrane protein, mitochondrial dynamin like GTPase (Opa1), outer membrane protein, Mitofusin 1 (Mfn1) and Mitofusin 2 (Mfn2) molecules are involved in mitochondrial fusion (Detmer and Chan, 2007; Friedman and Nunnari, 2014; Westermann et al., 2010). These processes are critical for maintenance of mitochondrial function, cellular quality control, and can play a role in neuronal plasticity and structure (Li et al., 2004; Cassidy-Stone et al., 2008; Chandra, Engeln, et al., 2017; Chen et al., 2007; Divakaruni et al., 2018). While most studies have not examined MSN subtype mitochondrial differences, recent studies are beginning to perform this analysis. For instance, D1 receptor signaling activation in NAc transiently facilitates mitochondrial function, by increasing respiration and ATP levels, which enhances social competitive behavior (van der Kooij et al., 2018). Additionally, we recently demonstrated that cocaine self-administration causes enhanced smaller length mitochondria, reflective of enhanced fission, in D1-MSN dendrites; which is consistent with increased Drp1 and fission promoting p(Ser616)-Drp1 in NAc D1-MSNs (Chandra, Engeln, et al., 2017). However, to date there is no investigation of MSN subtype mitochondria in basal conditions. Making use of control subjects used in our previous study we compared mitochondrial morphology between NAc MSN subtypes.

Materials and Methods

Animals

All animals used in this study follow guidelines set by the Institutional Animal Care and Use Committee (IACUC) at The University of Maryland School of Medicine. The health status of test animals was closely monitored by onsite veterinary staff and the experimenters throughout all experiments. All mice were maintained on a 12h light/dark cycle ad libitum food and water. Mice were group housed (3–5 per cage) for all experiments. D1-Cre hemizygote (Gensat, line Cat#5304499; RRID: MGI:5304499) Cat#5528384; RRID: MGI:5528384) FK150, or D2-Cre hemizygote (Gensat, line ER44, bacterial artificial chromosome (BAC) transgenic male mice from GENSAT (Gerfen et al., 2013; Gong et al., 2007) (www.gensat.org) on a C57BL/6J background were used for mitochondrial morphology study. Homozygous RiboTag (RT) mice on a C57BL/6J background, expressing a Cre-inducible HA-Rpl22 (Sanz et al., 2009) (The Jackson Laboratory, Cat#JAX029977; RRID: IMSR_JAX:029977) were crossed to D1-Cre or D2-Cre mouse lines to generate D1-

Cre-RT and D2-Cre-RT male mice (Chandra, Engeln, et al., 2017) and used for cell type-specific ribosome-associated mRNA isolation. Data from this study came from saline control mice used in Chandra, Engeln et al., 2017. Four male mice per group (D1-Cre and D2-Cre), age 10–12 weeks, were used for mitochondrial analysis. For RiboTag we used 12 D1-Cre-RT male mice, 8 D1-Cre-RT female mice, 12 D2-Cre-RT male mice, and 12 D2-Cre-RT female mice ages 8–10 weeks with 4 mice pooled per sample. Animal numbers were determined based on previous studies (Chandra et al., 2015; Chandra et al., 2017; Chandra, Engeln et al., 2017).

Mouse stereotaxic surgery

D1-Cre or D2-Cre mice (4 mice per group) were anesthetized using 4% isoflurane in a small induction chamber. After initial induction, isoflurane was maintained at 1–2% for the remainder of the surgery. Animals were placed in a stereotaxic instrument and their skull was exposed. 33 gauge Hamilton syringe needles were used to inject 0.2ul of AAV-DIO-mito-dsRed with AAV-DIO-ChR2-EYFP bilaterally into the NAc (anterior/posterior, AP +1.6; medial/lateral, ML $\pm$ 1.5; dorsal/ventral, DV-4.4, 10 degree angle) according to our previous studies (Chandra et al., 2015; Lobo et al., 2010). Mice were then returned to the vivarium for 2 weeks to allow for recovery and maximal virus expression, followed by saline self-administration as described in Chandra, Engeln et al., (2017. AAV-DIO-mito-dsRed virus was packaged in the lab as previously described (Chandra, Engeln et al., 2017. AAV2-DIO-ChR2-EYFP was obtained from University of North Carolina (UNC) Vector Core Facility.

RiboTag procedure

NAc samples were pooled from four D1-Cre-RT and D2-Cre-RT male and female mice and RNA isolation from immunoprecipitated polyribosomes using primary mouse anti HA antibody (Covance Research Products, Cat#MMS101R; RRID:AB_291262) and secondary antibody coated magnetic Dynabeads (Dynabeads protein G, Invitrogen) to pulldown the MSN specific RNA. Immunoprecipitated polyribosomes and non-immunoprecipitated input was prepared according to our previous studies (Chandra et al., 2015; Chandra et al., 2017; Chandra, Engeln et al., 2017). We used n=5 D1-Cre-RT and n=6 D2-Cre-RT samples. RNA from polyribosome immunoprecipitated samples or input was subsequently extracted using the Total RNA Kit (Omega) according to manufacturer's instructions. For cDNA synthesis, qRT-PCR and analysis we follow the steps as described previously (Chandra, Engeln et al., 2017; Chandra et al., 2015). The list of primers used in this study are Drp1-Forward GGGCACTTAAATTGGGCTCC, Drp1-Reverse TGTATTCTGTTGGCGTGAAC, Fis1-Forward GGCTGTCTCCAAGTCCAAATC, Fis1Reverse GGAGAAAAGGGAAGGCGATG, Opa1-Forward TCACCTCTGCGTTTATTTGAAGA, Opa1-Reverse GGGTAGAACGGGAGGAAAGG, Mfn1-Forward TATCGATGCCTTGCGGAGAT, Mfn1-Reverse GGCGAATCACAACACTTCCA, Mfn2-Forward GGAGACCAACAAGGACTGGA, Mfn2-Reverse TGCACAGTGACTTTCAACCG, Gapdh-Forward AGGTCGGTGTGAACGGATTG, Gapdh-Reverse TG TAGACCATGTAGTTGAGGTCA. Samples were run blinded to the condition. mRNA expression changes were measured using quantitative polymerase chain reaction (qPCR) with PerfeCTa SYBR Green FastMix (Quanta). Quantification of mRNA

changes was performed using the - CT method, using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a housekeeping gene and immunoprecipitated samples were then normalized to the averaged input from the respective MSN subtype.

Immunohistochemistry

To label the mitochondria we used dual virus combination of Cre-inducible adeno-associated viruses (AAV), AAV-DIO-mito-dsRed (to label mitochondria) and AAV-DIO-Chr2(H134)-EYFP (to label the soma and dendritic membranes) into the NAc of D1-Cre and D2Cre mice. D1-Cre and D2-Cre (receiving AAV2-DIO-Chr2-EYFP plus AAV2-DIO-mito-DsRed injections into NAc) mice were perfused with 0.1M phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA). Brains were immersed in azide PBS overnight. Brains were dissected with a vibratome (Leica) at 100 μ m into 0.1M PBS for mitochondria counting or sectioned on a cryostat (Leica). After washing with 0.1M PBS, regular immunostaining protocol was followed. Brain sections were blocked in 3% normal donkey serum with 0.3% Triton-X for 30 min at room temperature. Sections were then incubated overnight at room temperature in primary Aves Labs, Cat#GFP-1020; RRID: AB_10000240) antibody 1:4000 chicken anti-GFP (diluted in the 3% NDS and 0.3% tween 20 solution. On the second day, tissue sections were rinsed in 0.1M PBS followed by 1h incubation at room temperature in secondary antibodies, 1:1000 donkey anti-chicken-Alexa488 (Jackson ImmunoResearch cat# 703-545-155; RRID: AB_2340375). DsRed was not amplified via immunostaining, as endogenous virus expression was sufficient for imaging. Finally, sections were rinsed in PBS, mounted onto slides, and cover slipped.

Mitochondrial Imaging and Analysis

Detailed methods for mitochondrial imaging are described in (Chandra, Engeln et al., 2017). Sections were imaged on a Olympus Bx61 confocal microscope. A total of 6–12 cells were imaged per mouse by confocal scanning. High-resolution Z stacks images were obtained with 0.4mm increments using a 60x oil immersion objective with 2x digital zoom. Sections were scanned for neuron somas, proximal dendrites (within 50mm radius from soma), distal dendrites (over 100 mm radius from the soma), and distal secondary dendrites (the first nearest branch from distal dendrite). Using a three-dimensional (3D) image reconstruction which permits easy examination of cell structures and dendritic mitochondria morphology we analyzed multiple mitochondrial morphological parameters in NAc D1-MSNs and D2-MSNs. We examined mitochondria size in NAc D1-MSN and D2-MSN dendrites including proximal dendrites, within a 50 μ m radius from soma; distal dendrites, >100 μ m radius from the soma; and distal secondary dendrites, the first nearest branch from the distal dendrite. Image analysis for dendrite length and volume as well as mitochondrial number, length, and volume was quantified using Imaris 8.3 software (Bitplane; RRID: SCR_007370). Three metrics were used to assess mitochondrial morphology: length, density and index. Mitochondrial length was measured by using the BoundingBoxOO Length C setting that measures the length of the longest principal axis of the mitochondria. Mitochondrial length was analyzed as a relative frequency, comparing the proportion of mitochondria of each size range normalized to the total number of mitochondria in each dendrite. Mitochondrial density was measured by quantifying the total number of mitochondria per 10 μ m length of dendrite and is a way to examine mitochondrial number normalized per dendrite length.

Dendritic mitochondrial index was measured by quantifying the average mitochondrial length per 10 μm length of dendrite and is used as a metric for mitochondrial coverage within a neuronal process. Mitochondrial analysis was performed blinded to the condition.

Statistical analysis

Graphpad Prism 6.0 software was used for statistical analysis. Two-way ANOVA was used for mitochondrial size frequency analysis followed by Bonferroni post-hoc correction. Student's t-test was used for all other experiments. Comparisons were tested for normalcy. Significance was established when p-values were below 0.05. All graphs represent mean $\pm$ standard error (SEM). For qRT-PCR data, Grubbs outlier test was performed on data with obvious outliers and no more than one animal or sample was removed per group.

Key reagents

A table of key reagents with catalog numbers and RRIDs is included in supplementary information.

Results

With regard to mitochondrial length between neuronal subtypes, our data demonstrate an increase in the frequency of smaller length mitochondria in D2-MSN proximal dendrites, relative to D1-MSNs. (Figure 1b left panel; Two-way ANOVA: Interaction- $F_{(5, 36)}=5.264$, $p=0.001$, Bonferroni post hoc: $*p<0.05$). Mitochondria with 1.5–3.0 μm length were significantly higher in D2-MSN distal and distal secondary dendrites, while longer length mitochondria, with 3.0–4.5 μm length, were increased in D1-MSN distal (Figure 1b middle panel; Two-way ANOVA: Interaction- $F_{(5, 36)}=17.79$, $p<0.0001$, Bonferroni post hoc: $***p<0.001$), and distal secondary dendrites (Figure 1b right panel; Two-way ANOVA: Interaction- $F_{(5, 36)}=8.743$, $p<0.0001$, Bonferroni post hoc: $**p<0.01$, $***p<0.001$). Additionally, 4.5–6 μm mitochondria were increased in D1-MSN distal dendrites (Bonferroni post hoc: $*p<0.05$) and a trend of increased 0–1.5 μm length mitochondria was observed in D2-MSN distal dendrites (Bonferroni post hoc: $\#p=0.08$). Further, we did not observe any $>7.5\mu\text{m}$ mitochondria in D2-MSN distal secondary dendrites. These data demonstrate that overall mitochondrial length in D2-MSN dendrites is smaller compared to D1-MSNs.

To determine if the differences in mitochondrial length were NAc subregion specific we next analyzed mitochondrial morphology in NAc core and shell. In the NAc core (Figure 2a) mitochondria with $<1.5\mu\text{m}$ length were increased in D2-MSN proximal dendrites (Figure 2b; Two-way ANOVA- Interaction: $F_{(5, 36)}=3.672$, $p=0.009$, Bonferroni post hoc: $**p<0.01$). Larger mitochondria were not significantly different between NAc core MSN subtype proximal dendrites. In distal and distal secondary dendrites 1.5–3.0 μm sized mitochondria were increased in D2-MSNs, while 3.0–4.5 μm sized mitochondria were higher in D1-MSNs (Figure 2c, d; distal- Twoway ANOVA: Interaction- $F_{(5, 36)}=14.86$, $p<0.0001$, Bonferroni post hoc: $***p<0.001$; distal secondary- Two-way ANOVA: Interaction- $F_{(5, 36)}=4.806$, $p=0.002$, Bonferroni post hoc: $*p<0.05$). Further, larger mitochondria were not observed in NAc core D2-MSN distal ($>7.5\mu\text{m}$ mitochondria) and distal secondary ($>6.0\mu\text{m}$

mitochondria). These results mimic those seen previously with no anatomical subdivision, with smaller mitochondria in D2 MSNs and larger mitochondria in D1 MSNs.

In the NAc shell D1-MSNs and D2-MSNs (Figure 3a) mitochondrial size in proximal dendrites was similar between NAc shell MSN subtypes (Figure 3b; Two-way ANOVA: Interaction- $F_{(5, 36)}=1.133$, $p=0.36$). However, distal dendrites displayed a higher number of smaller sized mitochondria ($<1.5\mu\text{m}$) in NAc shell D2-MSNs (Figure 3c; Two-way ANOVA: Interaction- $F_{(5, 36)}=3.07$, $p=0.021$, Bonferroni post hoc: $*p<0.05$). While post-hoc analysis did not reveal a difference between NAc shell MSN subtype mitochondrial size, a significant interaction (MSN subtype x mitochondrial length) was observed (Two-way ANOVA: Interaction- $F_{(5, 35)}=3.105$, $p=0.0201$). The differences in mitochondrial length observed in the NAc shell follow the same trends but are not as pronounced as those seen in the NAc core.

Along with the frequency of mitochondrial size we assessed many other mitochondrial morphological parameters including mitochondrial density, mitochondrial index, and mitochondrial volume density across NAc MSN subtype dendrites, as well as mitochondrial volume in NAc MSN somas (Table 1). Mitochondrial index was higher in NAc core D1-MSN distal dendrites, however, other mitochondrial morphological properties were unchanged between NAc MSN subtypes.

Finally, to determine if the differences in the frequency of mitochondrial size between NAc D1-MSNs vs D2-MSNs may have a molecular basis we used D1-Cre-RiboTag (RT) and D2-Cre-RT mice, which allow for isolation of ribosome associated mRNA from NAc MSN subtypes. We examined ribosome-associated mRNA of mitochondrial fission and fusion molecules; *Drp1*, *Fis1* (fission) and *Opa1*, *Mfn1*, *Mfn2* (fusion). Both male and female mice were used for this analysis. We did not observe differences in gene expression between male and female mice (data not shown), therefore data were pooled for analysis. Ribosome-associated mRNA from each MSN subtype was normalized to GAPDH and then normalized to the averaged input isolated from the respective MSN subtype sample (Figure 4). Most fission and fusion ribosome-associated transcripts were unaltered between NAc MSN subtypes. However, a significant difference in *Opa1*, with higher expression in D1-MSNs compared to D2-MSNs, was observed. (Student's t-test: *Drp1* $t=1.57$, $df=9$, $p=0.15$; *Fis1* $t=0.21$, $df=9$, $p=0.84$; *Opa1* $t=4.13$, $df=9$, $p=0.002$; *Mfn1* $t=1.078$ $df=9$, $p=0.31$; *Mfn2* $t=0.27$, $df=9$, $p=0.79$).

Discussion

Our study identified distinct mitochondrial morphological differences between NAc D1-MSNs and D2-MSNs. Overall, we observe enhanced frequency of smaller length mitochondria across D2-MSN dendritic regions and enhanced larger mitochondria in D1-MSN dendrites. While these mitochondrial differences were observed in both core and shell, core dendrites exhibited these disparities to a greater extent. Many other mitochondrial morphological properties were unchanged between NAc MSN subtypes. However, mitochondrial index (total mitochondria length (μm)/10 μm length of dendrites) was

enhanced in D1-MSNs compared to D2-MSNs in NAc core distal dendrites, which likely reflects the differential size of mitochondria between these neuron subtypes.

When examining fission and fusion molecules in NAc MSN subtypes we observed enhanced levels of mRNA for a fusion molecule, Opa1, in D1-MSNs relative to D2-MSNs. This is consistent with our data showing increased longer mitochondria in D1-MSN dendrites. Enhanced Opa1 in D1-MSNs, relative to D2-MSNs, may underlie enhanced fusion in D1-MSN dendrites or reduced fusion, and consequently lesser larger and more smaller mitochondria, in D2-MSNs.

How these differences in mitochondrial size between NAc D1-MSN vs D2-MSN dendrites translate to neuronal function is unknown. Generally, mitochondrial size and function has been linked to neuronal signaling and output. Recent work in cortical projection neurons has shown that smaller mitochondrial size in axons is linked to higher synaptic Ca^{2+} , increased presynaptic release, and increased axonal branching, compared to conditions of reduced Drp1 expression and reduced fission (Lewis et al., 2018). The projection MSN subtypes in dorsal striatum and NAc display distinct electrophysiological properties, which could be related to their differences in dendritic mitochondrial morphology. D2-MSNs display increased sEPSC frequency in NAc core and shell (Ma et al., 2013), however, another study examining NAc MSN subtypes in prepubertal mice demonstrated reduced mEPSC frequency in D2-MSNs in NAc core (Willett et al., 2018). Increased smaller size mitochondria is associated with enhanced fission, which mediates structural and synaptic plasticity (Cassidy-Stone et al., 2008; Chandra, Engeln, et al., 2017; H. Chen et al., 2007; Divakaruni et al., 2018; Ishihara et al., 2009; Li et al., 2004). However, fission molecules, including Drp1 which is associated with enhanced structural and synaptic plasticity (Chandra, Engeln, et al., 2017; Divakaruni et al., 2018; Li et al., 2004), were similar between NAc MSN subtypes. Thus, the smaller mitochondria observed in D2 MSNs may represent reduced fusion as addressed above. D2-MSNs of NAc core, as well as dorsal striatum, also display enhanced intrinsic excitability (Ade et al., 2008; Cepeda et al., 2008; Chan et al., 2012; Chuhma et al., 2011; Gertler et al., 2008; Kreitzer & Malenka, 2007; Ma et al., 2013; Pascoli et al., 2014; Willett et al., 2018). This is consistent with less complex dendritic arbor in dorsal striatal and NAc D2-MSNs, compared to D1-MSNs (Gertler et al., 2008; Ma et al., 2012), since reduced dendritic arbor in NAc MSNs can underlie enhanced intrinsic activity (Francis et al., 2017). Interestingly, dendritic outgrowth in neurons is associated with increased mitochondrial fusion (Chen et al., 2007). Thus, increased Opa1 in D1-MSNs may drive enhanced fusion, reflective of larger mitochondria, mediating more complex dendritic architecture and reduced excitability in NAc D1-MSNs compared to D2-MSNs (Ma et al., 2012)

Most mitochondrial differences observed between D1 and D2 MSNs are specifically in mitochondrial length, while mitochondrial density and index remain comparable between cell types, apart from mitochondrial index in distal dendrites of the NAc Core. Both mitochondrial density and mitochondrial index represent ratios averaged over long stretches of dendrite, in contrast to mitochondrial length, which captures individual mitochondria. Cellular depolarization or inhibition can reduce or increase mitochondrial index, respectively (Li et al., 2004), indicating this metric is sensitive to neuronal activity. Indeed, in D1 MSNs

after cocaine self-administration, mitochondrial index in proximal dendrites is reduced (Chandra, et al., 2017). Individual mitochondria impact neuronal excitability via alterations in dendritic spine formation and maintenance (Li, et al., 2004; Divakaruni et al., 2018), which may be how differentially sized mitochondria contribute to the differences in baseline electrophysiological properties of NAc MSN subtypes.

In this study, D1- and D2-MSNs were identified using D1- and D2-Cre mouse lines, therefore, it is important to interpret these data considering the caveats associated with using genetic cell markers. Both the co-expression of D1 and D2 receptors in some NAc MSNs and the expression of D2 receptors in cholinergic interneurons may confound the results presented here. However, mitochondria were only analyzed in MSNs but that does not rule gene expression changes occurring in D2 expressing cholinergic interneurons.

Finally, our mitochondrial morphological analysis was performed only in male mice. While sex differences in MSN subtype electrophysiology properties are not observed in mice, at least during prepubertal ages (Willett et al., 2018), it will still be important to assess the mitochondrial properties in female subjects. Our RiboTag data was performed in a mixture of male and female mice, and we did not observe sex differences in gene expression, suggesting, that Opa1 mediated fusion and larger mitochondria could be present in female D1-MSNs. Future, studies examining mitochondria in females and basal and diseased states are needed including transgenerational studies since mitochondria are inherited through the mother.

Our data examining mitochondria in NAc MSNs in basal conditions is opposite to what is observed after cocaine exposure (Chandra, Engeln, et al., 2017). D1-MSNs of animals self-administering cocaine display enhanced smaller mitochondria across all dendritic regions and an increase in the Drp1 fission molecule relative to saline controls. In contrast D2-MSNs of cocaine self-administering mice have reduced smaller mitochondria and enhanced larger mitochondria in distal secondary dendrites, which is consistent with reduced Drp1 in these neurons. Our current data suggest that mitochondrial morphology in MSN subtypes have distinct basal identities, while stimuli such as repeated cocaine exposure can shift their mitochondrial states (Chandra, Engeln, et al., 2017). Collectively our study uncovers distinct mitochondrial morphological properties in MSN subtypes. By understanding these mitochondrial differences and the mechanisms underlying these basal mitochondrial states, it is plausible that such mechanisms could be targeted in diseased states that alter MSN mitochondria in attempts to shift them back to the basal state.

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Significance statement

The two main striatal neuron subtypes, known as medium spiny neurons (MSNs), often have distinct physiological and morphological adaptations at basal conditions and in diseased states. Recent studies indicate that mitochondria can play a role in these adaptations in diseased states. However, it is unclear if mitochondria are different between MSN subtypes in non-diseased states. In this study we demonstrate distinct mitochondrial morphological differences between these two MSN subtypes in ventral striatum, as well as a molecular change that could underlie these mitochondrial states. Insight into these basal mitochondrial states, could help to identify mechanisms that can be targeted in diseased states, with altered MSN mitochondria, in attempts to shift them back to the basal state.

Figure 1.

Quantification of mitochondrial length in NAc D1-MSNs and D2-MSNs. (a) Representative confocal images of a D1-MSN and a D2-MSN co-labeled with EYFP (green-dendrites and soma) and mito-dsRed (red- mitochondria). Scale bar: 50 μm . (b) D2-MSNs display an increase in the frequency of smaller length mitochondria in proximal (<1.5 μm), distal (1.5–3 μm) and distal secondary dendrites (1.5–3 μm) in comparison with D1-MSNs. D1-MSNs display an increase in larger length mitochondria in distal (3.0–4.5 μm) and distal secondary dendrites (3.0–4.5 μm). # $p<0.10$ * $p<0.05$, ** $p<0.01$, *** $p<0.001$; error bars, SEM

Figure 2.

Mitochondrial length in NAc core D1-MSN and D2-MSN dendrites. (a) Coronal section highlighting the NAc core, the region that MSN mitochondria were quantified. (b-d) Representative confocal images of D1-MSN and D2-MSN dendrites co-labeled with EYFP (green-dendrites) and mito-dsRed (red- mitochondria). The bottom panels display a heatmap of mitochondrial length in MSNs dendrites. Scale bar: 5µm. (b-d) The frequency of smaller length mitochondria is enhanced in D2-MSN proximal (<1.5µm), distal (1.5–3.0µm) distal secondary (1.5–3.0µm) dendrites in comparison with D1-MSNs. D1-MSNs display an increase in the frequency of larger mitochondria in distal 3–4.5µm and distal secondary dendrites (3–4.5µm) relative to D2-MSNs. *p<0.05, **p<0.01, ***p<0.001; error bars, SEM.

Figure 3. Mitochondrial length in NAc shell D1-MSN and D2-MSN dendrites. (a) Coronal section highlighting the NAc shell region that MSN mitochondria were quantified. (b-d) Representative confocal images of D1-MSN and D2-MSN dendrites co-labeled with EYFP (green- dendrites) and mito-dsRed (red- mitochondria). The bottom panel displays a heatmap of mitochondrial length in MSNs dendrites. Scale bar: 5µm. (b-d) D2-MSNs display an increase in the frequency of smaller mitochondria (<1.5µm) in distal dendrites relative to D1-MSNs. *p<0.05; error bars, SEM

Figure 4.

NAc MSN subtype ribosome-associated mRNA levels of mitochondrial fission and fusion molecules. Opa1 ribosome-associated mRNA is differentially expressed in NAc D2-MSNs vs D1-MSNs. ** $p < 0.01$; error bars, SEM. qPCR data normalized with input samples shown as dashed line.

Table 1.

Mitochondrial morphological properties in NAc D1-MSNs and D2-MSNs.

Neuron Compartment	Mitochondrial properties	NAc region	D1-MSN	D2-MSN	p-value
Soma (n=4each)	Volume (μm^3)	Total NAc	0.14 $\pm$ 0.03	0.13 $\pm$ 0.03	0.70
		NAc Core	0.14 $\pm$ 0.02	0.15 $\pm$ 0.03	0.95
		NAc Shell	0.15 $\pm$ 0.02	0.14 $\pm$ 0.045	0.81
Proximal dendrite (n=4 each)	Mitochondrial density (Number of mitochondria/10 μm length of dendrites)	Total NAc	2.77 $\pm$ 0.59	2.52 $\pm$ 0.24	0.71
		NAc Core	2.65 $\pm$ 0.42	2.63 $\pm$ 0.17	0.96
		NAc Shell	2.95 $\pm$ 0.73	2.45 $\pm$ 0.31	0.55
	Mitochondrial Index (Total mitochondria length (μm)/10 μm length of dendrites)	Total NAc	0.80 $\pm$ 0.11	0.62 $\pm$ 0.10	0.29
		NAc Core	0.66 $\pm$ 0.08	0.47 $\pm$ 0.09	0.19
		NAc Shell	0.82 $\pm$ 0.15	0.67 $\pm$ 0.15	0.51
	Mitochondrial volume density (Total mitochondria volume (μm^3)/total dendrites volume(μm^3))	Total NAc	1.00 $\pm$ 0.24	1.00 $\pm$ 0.18	0.99
		NAc Core	1.00 $\pm$ 0.31	1.00 $\pm$ 0.23	0.99
		NAc Shell	1.00 $\pm$ 0.16	0.98 $\pm$ 0.35	0.95
Distal dendrites (n=4 each)	Mitochondrial density (Number of mitochondria/10 μm length of dendrites)	Total NAc	2.50 $\pm$ 0.18	3.00 $\pm$ 0.26	0.18
		NAc Core	2.50 $\pm$ 0.18	3.20 $\pm$ 0.27	0.08
		NAc Shell	2.40 $\pm$ 0.21	2.75 $\pm$ 0.24	0.35
	Mitochondrial Index (Total mitochondria length (μm)/10 μm length of dendrites)	Total NAc	0.45 $\pm$ 0.05	0.35 $\pm$ 0.03	0.13
		NAc Core	0.46 $\pm$ 0.03	0.35 $\pm$ 0.03	0.03
		NAc Shell	0.48 $\pm$ 0.04	0.37 $\pm$ 0.03	0.07
	Mitochondrial volume density (Total mitochondria volume(μm^3)/total dendrites volume(μm^3))	Total NAc	0.97 $\pm$ 0.17	1.00 $\pm$ 0.11	0.90
		NAc Core	0.97 $\pm$ 0.26	97.00 $\pm$ 0.13	0.99
		NAc Shell	1.00 $\pm$ 0.12	1.00 $\pm$ 0.11	0.99
Distal secondary dendrites (n=4 each)	Mitochondrial density (Number of mitochondria/10 μm length of dendrites)	Total NAc	2.70 $\pm$ 0.17	2.80 $\pm$ 0.12	0.73
		NAc Core	2.62 $\pm$ 0.10	2.85 $\pm$ 0.11	0.20
		NAc Shell	2.70 $\pm$ 0.22	2.70 $\pm$ 0.32	0.99
	Mitochondrial Index (Total mitochondria length (μm) /10 μm length of dendrites)	Total NAc	0.45 $\pm$ 0.29	0.42 $\pm$ 0.05	0.67
		NAc Core	0.43 $\pm$ 0.03	0.40 $\pm$ 0.04	0.61
		NAc Shell	0.48 $\pm$ 0.05	0.45 $\pm$ 0.03	0.54
	Mitochondrial volume density (Total mitochondria volume (μm^3)/total dendrites volume(μm^3))	Total NAc	0.99 $\pm$ 0.26	0.97 $\pm$ 0.13	0.94
		NAc Core	1.00 $\pm$ 0.38	1.00 $\pm$ 0.29	0.99
		NAc Shell	1.00 $\pm$ 0.21	1.00 $\pm$ 0.11	0.99

Key reagents table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Chicken anti GFP	Aves Labs	Cat#GFP-1020; RRID: AB_10000240
Mouse anti HA	Covance Research Products	Cat#MMS101R; RRID:AB_291262
Dynabeads protein G	Invitrogen	Cat#10004D
Donkey anti chicken Alexa Fluor 488 2 nd antibody	Jackson Immuno Research	Cat#703-545-155; RRID: AB_2340375
Bacterial and Virus Strains		
AAV2-EF1a-DIO-mito dsRed	Lobo lab, University of Maryland School of Medicine; Chandra et al. Neuron, 2017	N/A
AAV2-EF1a-DIO-hChR2(H134R)-EYFP	University of North Carolina Vector Core facility	N/A
Chemicals, Peptides, and Recombinant Proteins		
Cocaine HCl	Sigma-Aldrich	Cat#C5776, CAS5321-4
Critical Commercial Assays		
MicroElute Total RNA kit	Omega bio-tek	Cat#R6831_01
iScript cDNA synthesis kit	Biorad	Cat#1708890
PerfeCTa SYBR green FastMix	Quanta bio	Cat#95072-250
Deposited data		
N/A	N/A	N/A
Experimental Models: Organisms/Strains		
Mouse: D1-Cre (line FK150) BAC transgenic on a C57BL/6J background	Gensat	Cat#5528384; RRID: MGI:5528384
Mouse: D2-Cre (line ER44) BAC transgenic on a C57BL/6J background	Gensat	Cat#5304499; RRID: MGI:5304499
Mouse: RiboTag: Rpl22 ^{tm1.1P_{sam}} on a C57BL/6J background	The Jackson Laboratory	Cat#JAX029977; RRID: IMSR_JAX:029977
Oligonucleotides		
For all primer mouse sequences, please see in method	This paper	N/A
Software and Algorithms		
GraphPad Prism 6.0	GraphPad	N/A
Imaris 8.2	Bitplane	RRID:SCR_007370
FluoView FV500	Olympus	N/A
Other		