



Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: [www.elsevier.com/locate/schres](http://www.elsevier.com/locate/schres)

## Postmortem studies on mitochondria in schizophrenia

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### ARTICLE INFO

#### Article history:

Received 27 September 2016

Received in revised form 27 January 2017

Accepted 30 January 2017

Available online xxx

#### Keywords:

Postmortem

Psychosis

Electron microscopy

Neuropathology

Cytochrome oxidase

### ABSTRACT

The aim of this paper is to provide a brief review of mitochondrial structure as it relates to function and then present abnormalities in mitochondria in postmortem schizophrenia with a focus on ultrastructure. Function, morphology, fusion, fission, motility,  $\Delta\Psi_{\text{mem}}$ , ATP production, mitochondrial derived vesicles, and mitochondria-associated ER membranes will be briefly covered. Pathology in mitochondria has long been implicated in schizophrenia, as shown by genetic, proteomic, enzymatic and anatomical abnormalities. The cortex and basal ganglia will be reviewed. In the anterior cingulate cortex, the number of mitochondria per neuronal somata in layers 5/6 in schizophrenia is decreased by 43%. There are also fewer mitochondria in terminals forming axospinous synapses. In the caudate and putamen the number of mitochondria is abnormal in both glial cells and neurons in schizophrenia subjects, the extent of which depends on treatment, response and predominant lifetime symptoms. Treatment-responsive schizophrenia subjects had about a 40% decrease in the number of mitochondria per synapse in the caudate nucleus and putamen, while treatment resistant cases had normal values. A decrease in mitochondrial density in the neuropil distinguishes paranoid from undifferentiated schizophrenia. The appearance, size and density of mitochondria were normal in the nucleus accumbens. In the substantia nigra, COX subunits were affected in rostral regions. Mitochondrial hyperplasia occurs within axon terminals that synapse onto dopamine neurons, but mitochondria in dopamine neuronal somata are similar in size and number. In schizophrenia, mitochondria are differentially affected depending on the brain region, cell type, subcellular location, treatment status, treatment response and symptoms.

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### 1. Introduction

The aim of this paper is to provide a brief review of mitochondrial structure as it relates to function and then present abnormalities in mitochondria in schizophrenia with a focus on ultrastructure.

#### 1.1. Normal mitochondrial function

Mitochondria are famous for producing 95% of cellular energy using the electron transport chain (Wong-Riley, 1989). In addition, they are necessary for other cellular functions including intracellular calcium buffering (Babcock and Hille, 1998; Duchen et al., 2008; Gunter et al., 1994), production of reactive oxygen species (Chang and Reynolds, 2006), regulation of apoptosis (Susin et al., 1999) and modulation of synaptic activity (Duchen et al., 2008; Li et al., 2004; Miller and Sheetz, 2004; Sheng and Cai, 2012). Mitochondria are plastic and dynamic organelles that can change shape, location, size and number in response to energy demands (Isaacs et al., 1992; Ligon and Steward, 2000; Mjaatvedt and Wong-Riley, 1988; Prince et al., 1999).

#### 1.2. The afterlife of mitochondria

Neuronal function in the brain requires energy in the form of ATP. To assess mitochondrial activity in human brain, investigators have previously utilized frozen postmortem brain tissue to analyze mitochondrial enzymatic activities (Devi et al., 2008), protein levels (Park et al., 2001), and DNA (Alam et al., 1997; Vila et al., 2008). However, there are several other vital indices that provide insight into brain mitochondrial activity which cannot be accomplished in frozen tissue samples. These include measurements of the mitochondrial membrane potential ( $\Delta\Psi_{\text{mem}}$ ), ATP production, calcium buffering capacity, and respiration, which together give an overall assessment of mitochondrial health and activity. For example, the  $\Delta\Psi_{\text{mem}}$ , which is the electrochemical gradient across the inner mitochondrial membrane, serves as an important overall indicator of mitochondrial activity. It is also a fundamental component of respiring mitochondria. The  $\Delta\Psi_{\text{mem}}$  is linked to many crucial mitochondrial functions including ATP synthesis, calcium homeostasis, mitochondrial protein import, and mitochondrial metabolite transport (Hüttemann et al., 2008), all of which are typically analyzed in real-time measurements.

Although metabolic activity in the brain ceases at death, we have been able to measure the  $\Delta\Psi_{\text{mem}}$  and ATP production in mitochondria isolated from human postmortem brains with postmortem intervals of

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up to 8.5 h (Barksdale et al., 2010). Furthermore, postmortem brain mitochondria retain their  $\Delta\Psi_{\text{mem}}$  and ATP production capacities following cryopreservation, indicating that functional isolated mitochondria can be archived for future studies. The findings that  $\Delta\Psi_{\text{mem}}$  and ATP generation can be reinitiated in brain mitochondria hours after death indicates that postmortem brains can be an abundant source of viable mitochondria for the study of metabolic processes in health and disease, and it is also possible to archive these mitochondria for future studies (Barksdale et al., 2010).

### 1.3. Morphology

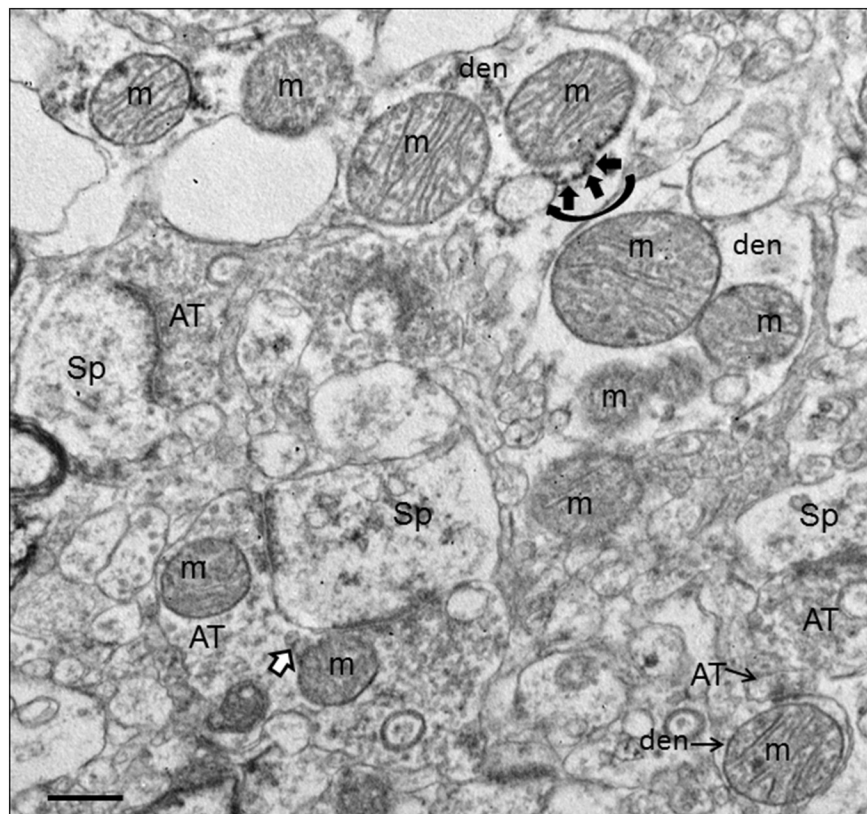
Mitochondria have different shapes; they can be round, elongated, blob-shaped, donut-shaped or have an elaborate configuration (Picard and McEwen, 2014). In most instances the overall shape of the mitochondrion has functional implications (Youle and van der Bliek, 2012; Ahmad et al., 2013). Take for example the relationship between the shape of mitochondria and the production of reactive oxygen species. In cell culture, mitochondrial stressors can induce the conversion of straight (i.e. rod-shaped) mitochondria to donut-shaped mitochondria, to blob-shaped mitochondria (Liu and Hajnóczky, 2011; Ahmad et al., 2013). Blob-shaped mitochondria generate the highest levels of reactive oxygen species, followed by donut shaped compared to straight mitochondria (Liu and Hajnóczky, 2011; Ahmad et al., 2013). While donut-shaped mitochondria can revert back to the straight configuration, blob-shaped mitochondria are unable to revert back to healthier configurations. In axon terminals, donut-shaped mitochondria are associated with shorter synapses and fewer docked vesicles; in the dorsolateral prefrontal cortex donut-shaped mitochondria are correlated with poor delayed response memory (Hara et al., 2014).

In addition to shape, the morphology of the cristae, matrix and inner mitochondrial membrane correspond to the activity of the electron transport chain (Hackenbrock, 1968). At the ultrastructural level, mitochondria have an orthodox or condensed configuration, which corresponds to high or low energy producing states, respectively (Hackenbrock, 1968). In the condensed configuration, the matrix is smaller and denser, the inner membrane is irregularly organized and forms few cristae, and the space between inner and outer membranes is increased. The orthodox configuration is what is usually illustrated in electron micrographs (Figs. 1 and 2).

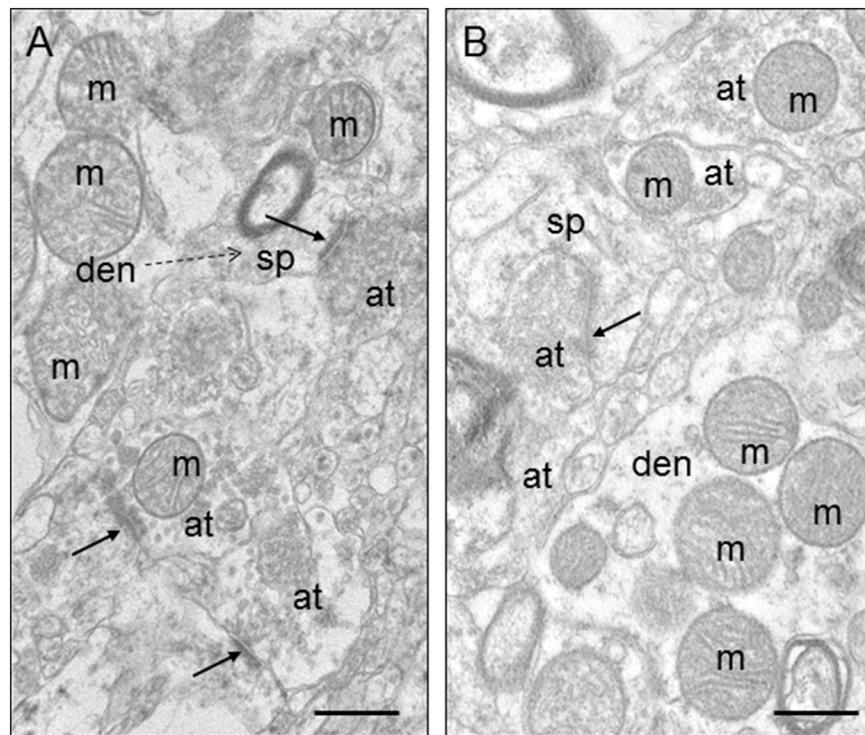
In the aging nervous system, there are reports of fewer mitochondria, but they are larger in size (Shigenaga et al., 1994; Soghomonian et al., 2010; Martinelli et al., 2006). Perhaps enlargement of the mitochondria is a compensatory mechanism to deal with the decreased number. However, fewer bigger mitochondria are able to meet short energy demands, but sustained energy demands are not met (Shigenaga et al., 1994; Soghomonian et al., 2010; Martinelli et al., 2006). Thus, the examination of size and shape of mitochondria will reveal important information about their functionality.

### 1.4. Fission and fusion

Mitochondria function as a dynamic network constantly undergoing fission and fusion, the balance of which is important in maintaining their structural integrity and function (Legros et al., 2002). Proteins that cause mitochondrial fusion include mitofusin-1, mitofusin-2 and Opa1 (Koshiba et al., 2004). Although they are critical for mitochondrial fusion, they are necessary for other functions as well. For example, besides its role in mitochondrial fusion, mitofusin-2 contributes to the maintenance and operation of the mitochondrial network (Bach et al., 2003). Mitofusin 2 is also necessary for transporting mitochondria and



**Fig. 1.** Electron micrograph of human striatum. Mitochondria (m) are shown in various subcellular locations. In the dendrite (den) at the top of the field, mitochondrial associated ER (MAM) is shown (curved black arrow) with ER (short black arrows) connecting to the adjacent mitochondrion. In the terminal (AT) synapsing on a spine (Sp) in the lower part of the field, mitochondrial derived vesicles (MDVs) are shown (white arrow with black outline) budding off of a mitochondrion. Scale bars = 0.5  $\mu\text{m}$ . Figure is modified from Fig. 2a in Somerville et al., 2011b.



**Fig. 2.** Electron micrographs of human striatum. A) Control. B) Schizophrenia. Mitochondria (m) are shown in various subcellular locations. Abbreviations: s, spine; at, axon terminal; den, dendrite; ma, myelinated axon; solid arrows, synapses; dotted arrow, spine emerging from dendrite. Scale bars = 0.5  $\mu$ m. Figure is modified from Fig. 1 in Somerville et al., 2011b.

proper localization in neuronal processes (Misko et al., 2010; Sheng and Cai, 2012). Mitochondria undergo fragmentation in response to various stimuli, including electron transport chain toxins and mitochondrial DNA-linked mutations. Dynamin related protein 1 (Drp1) (Smirnova et al., 2001) interacts with other proteins—mitochondrial fission protein 1 (Fis1) (James et al., 2003), mitochondrial fission factor (Mff) (Otera et al., 2010), and mitochondrial dynamics proteins MiD49 and MiD51 (recently reviewed by Bertholet et al., 2016)—to cause division of mitochondria. Mitochondrial division occurs at ER contact sites on mitochondrial constrictions (de Brito and Scorrano, 2010; Friedman et al., 2011). While both Fis1 and Mff have roles in mitochondrial fission, either MiD49 or MiD51 can mediate Drp1 recruitment and mitochondrial fission in the absence of Fis1 and Mff (Losón et al., 2013). Therefore, there are multiple proteins that are sufficient, but not necessary, to recruit Drp1 to mediate mitochondrial fission.

### 1.5. Mitochondria derived vesicles

Mitochondria derived vesicles (MDVs) are structures that bud off of mitochondria and transport damaged cargo to peroxisomes or lysosomes (Fig. 1). MDVs have either single or double membranes, unique densities and uniform diameter (Soubannier et al., 2012). MDVs are stimulated upon various forms of mitochondrial stress, and the vesicles incorporate cargo, whose composition depends upon the type of stress. Stress-induced MDVs are selectively enriched for oxidized proteins, suggesting that conformational changes induced by oxidation may initiate their incorporation into the vesicles.

### 1.6. Mitochondria-associated endoplasmic reticulum membranes

Mitochondria are connected to the endoplasmic reticulum via mitochondria-associated endoplasmic reticulum membranes (MAMs; Hayashi et al., 2009) (Fig. 1). MAMs are subregions of the endoplasmic reticulum that have a unique lipid composition, are enriched in cholesterol and anionic phospholipids, and have characteristics of lipid rafts

(Hayashi and Fujimoto, 2010). MAMs are involved in a number of key metabolic functions, including phospholipid and cholesterol metabolism (Hayashi et al., 2009). MAMs are also enriched in proteins related to the control of mitochondrial division (Friedman et al., 2011) and dynamics (Schon and Area-Gomez, 2013). Mitofusin 2 tethers endoplasmic reticulum to mitochondria (de Brito and Scorrano, 2008). Defects in MAM-localized proteins and/or disturbances in MAM function play a role in neurodegenerative diseases (Area-Gomez et al., 2012; Schon and Przedborski, 2011; Ottolini et al., 2013).

### 1.7. Motility

In neurons, biogenesis of mitochondria occurs in the cell soma (Davis and Clayton, 1996) and they have a half-life of functionality of about one month (Menzies and Gold, 1971). Healthy mitochondria have relatively high membrane potentials and 90% of them move anterogradely, suggesting that healthy mitochondria are trafficked away from their point of origin in the cell soma (Miller and Sheetz, 2004). As mitochondria move down a neuronal process, they stop and start; at least half of mitochondria are stationary, whereas the rest move at variable speeds (Allen et al., 1982; Hollenbeck, 1996; Hollenbeck and Saxton, 2005; Ligon and Steward, 2000). Mitochondria are more stationary at synaptic sites, where the energy demand is especially high (Li et al., 2004; Chang et al., 2006). Mitochondria move along microtubules via kinesin and adaptors for anterograde transport and via dynein and adaptors for retrograde transport; they also can be anchored via actin and neurofilaments (reviewed by Lin and Sheng, 2015).

Axonal transport happens at different speeds depending on the particular substance being transported, its functional role, and the direction of transport (Niescier et al., 2016). In axons, a large range of average velocities for mitochondria have been reported for both anterograde and retrograde movement, probably due to sampling different lengths of axon, and/or different distances from the cell body (MacAskill and Kittle, 2010). It has been classically thought that at the end of their functional life span mitochondria are degraded by autophagy, or

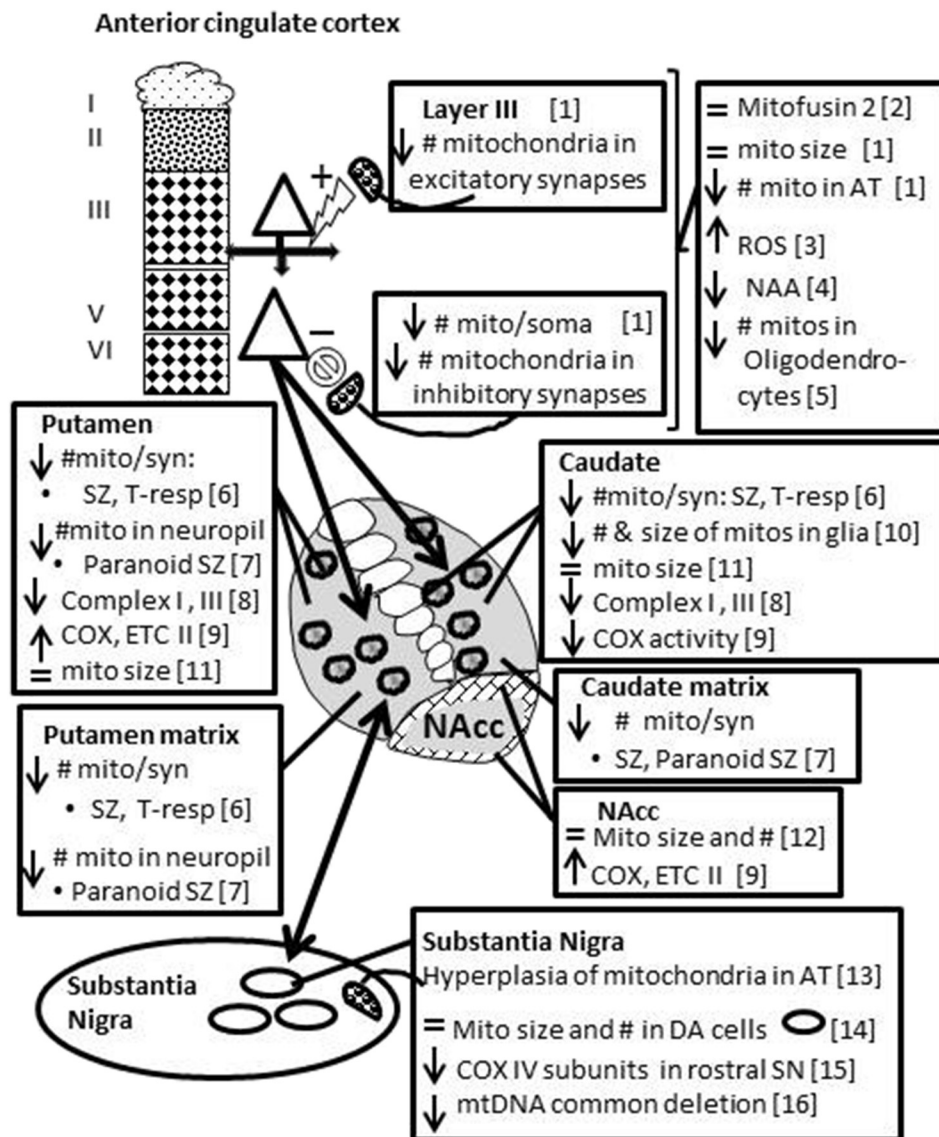


retrogradely transported back to the cell soma and recycled there (reviewed by Ploumi et al., 2016). New evidence challenges the notion that mitochondria go back to the cell body to die (Niescier et al., 2016). Only a few studies to date have examined transport of mitochondria in the entire axon and over a long period of time (O'Toole et al., 2008; Niescier et al., 2016). Niescier et al. (2016) showed that mitochondria originating from axon terminals moving retrogradely never actually make it all the way back to the cell body, and only a few mitochondria moving anterogradely out of the cell soma ever arrive at the axon terminals. Importantly, the velocity of mitochondria varies depending on the location in the axon, so examining the entire axon becomes very important to obtain average mitochondrial speeds. While it was classically thought that the rate of anterograde transport in the axon was independent of electrical activity (reviewed by Oztas, 2003), new evidence indicates otherwise (Sajic et al., 2013).

## 2. Mitochondrial abnormalities in schizophrenia

Among the many abnormalities in schizophrenia are those related to mitochondrial function (for reviews see Ben-Shachar, 2002; Ben-Shachar and Laifenfeld, 2004; Clay et al., 2011). Evidence of mitochondrial dysfunction in schizophrenia includes genetic (Marchbanks et al., 2003; Kvafo et al., 2008; Verge et al., 2011), metabolic (Prabakaran et al., 2004), enzymatic (Prince et al., 1999, 2000; Maurer et al., 2001) and anatomical abnormalities (reviewed herein). The production of ATP and calcium buffering are essential in maintaining synaptic strength and abnormalities in these processes could lead to decreased metabolism and defective synaptic activity (Ben-Shachar and Laifenfeld, 2004; Chang and Reynolds, 2006; Duchen et al., 2008).

Many of our electron microscopic studies and those of Uranova and colleagues (Kolomeets and Uranova, 1999; Uranova et al., 1996, 2001,



**Fig. 3.** Schematic drawing of the cortical striatal nigral pathway showing mitochondrial abnormalities in each region in schizophrenia. Depending on the study, the entire structure or subdivisions within, were studied. The cortex is divided in layers for layer specific changes. Patch (circles) and matrix compartments are shown for the caudate and putamen when abnormalities are striosome or matrix specific. The core and shell (hatched) of the nucleus accumbens are depicted. Mito, mitochondria. ROS, reactive oxygen species. NAA, N-acetylaspartate. COX, cytochrome oxidase. ETC, electron transport chain. Mito/synapse, mitochondria per terminal forming a synapse. T-resp, treatment responsive schizophrenia. AT, axon terminals. DA, dopamine. mtDNA, mitochondrial DNA. 1) Roberts et al., 2015; 2) Barksdale et al., 2014; 3) Wang et al., 2009; 4) Reid et al., 2010; 5) Uranova et al., 2007, Vikhrev et al., 2016; 6) Mamdani et al., 2014; 7) Somerville et al., 2011a, 2011b; 8) Somerville et al., 2012; 9) Maurer et al., 2001; Ben-Shachar and Karry, 2008; 10) Cavelier et al., 1995; Prince et al., 1999, 2000; 11) Uranova et al., 1996, 2001; 12) Kung and Roberts, 1999; 13) McCollum et al., 2015; 14) Kolomeets and Uranova, 1999; 15) Walker and Roberts, 2016; 16) Rice et al., 2014.

2007; Vikhrev et al., 2016) have examined mitochondrial size, number, location, structural integrity and markers of viability in various brain regions implicated in schizophrenia. Ultrastructural studies are possible with well-preserved tissue with short postmortem intervals (Fig. 2). Here mitochondrial abnormalities in structure and function in schizophrenia are reviewed in several, but not all, brain regions (see Fig. 3 for a summary).

### 2.1. Anterior cingulate cortex

The anterior cingulate cortex (ACC), a structurally and functionally diverse region, is one of several brain regions that are abnormal in schizophrenia (Fornito et al., 2009). The ACC serves to regulate several cognitive functions including reward anticipation, emotion, empathy, and decision making. Previous imaging and electrophysiological studies have revealed changes in the ACC related to psychosis and antipsychotic drug response (Lahti et al., 2006, 2009). *N*-acetylaspartate (NAA), a marker of neuronal integrity, is decreased in the ACC in schizophrenia (Reid et al., 2010). NAA and Glx (a measure of glutamate) are not correlated in schizophrenia, but are in controls (Kraguljac et al., 2012). In vivo imaging has shown decreased BOLD during memory retrieval (Hutcheson et al., 2012). Oxidative stress, a result of mitochondrial production of reactive oxygen species, is increased (Wang et al., 2009). In a recent study markers of synapses and mitochondria were examined in the ACC in a schizophrenia cohort divided by treatment response (Barksdale et al., 2014). Protein levels of the mitochondrial marker, mitofusin-2, were normal in schizophrenia cases; moreover, there were no effects of treatment response. Normal protein levels suggest that mitochondrial fusion and maintenance and operation of the mitochondrial network may be intact. However, while mitofusin 2 may be normal, there are many other mitochondrial proteins involved in fusion that might be abnormal.

In an ultrastructural study, the number of mitochondria per neuronal somata in schizophrenia was decreased by 43% of that of controls (Roberts et al., 2015). This was due to a selective loss in layers 5/6. The density of mitochondria in the neuropil (connections in between cell bodies) was similar between controls and the schizophrenia cases in the combined ACC (layers 3,5,6) as well as the separate analysis of layer 3 and layers 5/6. There were no differences in size of mitochondria in the neuronal somata or in the neuropil. Moreover, there were no obvious blob or donut shaped mitochondria. This is somewhat surprising considering the observed increase in reactive oxygen species (Wang et al., 2009), which are produced at a higher rates in blob and donut shaped mitochondria (Liu and Hajnóczky, 2011; Ahmad et al., 2013), yet only round or rod shaped mitochondria were observed.

The number of mitochondria in axon terminals in the combined ACC was lower in schizophrenia as compared to controls. There were fewer mitochondria in certain subtypes of terminals, especially in terminals forming axospinous synapses. Interestingly, in Layer 3, mitochondria were less frequent in *asymmetric* axospinous synapses, while in layers 5/6 there were fewer mitochondria in *symmetric* axospinous synapses. Asymmetric synapses (also called Gray Type I) are excitatory, while symmetric synapses (also called Gray Type II) are inhibitory (Gray, 1959). In layer 3 of non-human primates, asymmetric axospinous synapses are formed by afferents from the medial dorsal thalamus, contralateral ACC and other intracortical connections (see Hoftman et al., 2016). Symmetric axospinous synapses in deep cortical layers originate in part from dopamine connections (Kubota et al., 2016). In other ultrastructural studies, Uranova and Aganova (1989) have shown layer specific abnormalities such as fewer synapses in the ACC, and fewer mitochondria in oligodendrocytes in both gray and white matter (Uranova et al., 2007; Vikhrev et al., 2016). The latter observation suggests that oligodendrocytes need or have less energy, which may have an impact on proper myelination.

Fewer mitochondria in axon terminals suggest a decrease in efficacy of synaptic transmission (Brodin et al., 1999; Verstreken et al., 2005;

Hall et al., 2012), and in the case of the ACC this would affect both excitatory and inhibitory connections. Pyramidal neurons in layers 5/6 project to the striatum or brainstem, or thalamus (Goldman and Nauta, 1977). The reduction of mitochondria per soma in the deep layers suggests that neurons that project to these subcortical regions may have compromised metabolism. The layer specific location suggests abnormalities in multiple connections that may have effects within the cortex as well as in several downstream pathways. The decrease in NAA and increase in oxidative stress may be reflected by decreased numbers of mitochondria, rather than smaller size or irregular shapes.

### 2.2. Dorsal striatum

#### 2.2.1. Treatment status

Several studies have shown mitochondrial abnormalities in the striatum in subjects with schizophrenia such as decreases in complex I and III activity, protein and/or mRNA levels (Ben-Shachar and Karry, 2008; Maurer et al., 2001), and changes in complex IV (Cavelier et al., 1995; Prince et al., 1999, 2000). The complexes do not necessarily change in the same direction in all nuclei. For instance, there is a decrease in COX activity in the caudate and an increase in COX and succinate dehydrogenase in the putamen in SZ postmortem tissue (Prince et al., 1999). Most of these changes appear to be caused by antipsychotic drugs (APDs) (Burkhardt et al., 1993; Prince et al., 1997).

Previous ultrastructural studies in the striatum in schizophrenia have shown similar numbers (Somerville et al., 2011a) and size (Kung and Roberts, 1999) of mitochondria. However, decreases in the number of mitochondria per synapse were detected in both the caudate and putamen in schizophrenia. Since the majority of mitochondria are in dendrites, fewer mitochondria in axon terminals may be missed in overall neuropil counts. Further analysis showed that subjects divided by treatment status into off drug, atypical APD or typical APD, all showed significant decreases in the putamen (Somerville et al., 2011a). Since the schizophrenia subgroups on APD had similar decreases in mitochondrial number compared to the off-drug subjects, this result may not be an APD effect. However, haloperidol, a typical APD, does reduce the number of mitochondria in the striatum of chronically treated rats (Roberts et al., 1995).

When examining patch matrix compartments, striatal compartments associated with different circuitry and function (Graybiel and Ragsdale, 1978), only the matrix exhibited changes. The matrix compartment preferentially receives inputs from the dorsolateral prefrontal cortex (Eblen and Graybiel, 1995), which processes higher cognitive functions, such as working memory (Goldman-Rakic, 1999). Motor and somatosensory cortices also project to the matrix in a topographical fashion (Flaherty and Graybiel, 1993). Thus, it would be expected that mitochondrial abnormalities in the striatal matrix could impact cognition, working memory, motor, and somatosensory functions.

Mitochondria are also affected in glial cells. In the caudate, they are decreased in number in astrocytes (Uranova et al., 1996) and are smaller in oligodendroglia (Uranova et al., 2001). Either of these results could compromise the function of these cell types.

#### 2.2.2. Symptoms

Symptoms of schizophrenia can vary markedly between patients, and similar symptoms may be related to shared brain pathophysiology. In addition, a relationship between symptoms and mitochondrial pathology is evident in blood. Lymphocytes analyzed from paranoid schizophrenia patients and controls showed less mitochondrial volume in patients in certain types of lymphocytes (Uranova et al., 2007). Moreover, the severity of the mitochondrial deficit was positively correlated with symptom severity, linking paranoid symptoms with mitochondrial impairment, albeit in blood (Uranova et al., 2007). Also, COX and complex II have been shown to correlate with the severity of symptoms in the putamen (Prince et al., 2000), again linking symptoms with mitochondrial dysfunction.

In an ultrastructural study of the caudate and putamen, a decrease in the density of mitochondria in the neuropil was observed in the paranoid group compared to both the controls and the undifferentiated group (Somerville et al., 2012). In addition, the putamen of the paranoid group had fewer mitochondria in axon terminals compared to the NCs and the undifferentiated group. However, in neither the caudate nor the putamen was the number of mitochondria per synapse differentially decreased in either subgroup. When examining patch matrix compartments, only the matrix exhibited changes. These results could be associated with the symptoms of paranoia and/or could represent a protective mechanism against some of the symptoms that are less pronounced in this subtype than in the undifferentiated subgroup, such as cognitive and emotional deficits.

### 2.2.3. Treatment response

One third of patients with schizophrenia do not respond to medication and remain psychotic (Meltzer, 1997). The patients that do respond, do so on a continuum, but only respond to positive symptoms. Cognitive and negative symptoms are poorly treated in all people with schizophrenia. Although treatment response and resistance have a biological basis (Sheitman and Lieberman, 1998), all studies conducted outside of our own work have been imaging live people. In a cohort of subjects rated for treatment response or resistance, treatment-responsive schizophrenia subjects had about a 40% decrease in the number of mitochondria per synapse in the caudate nucleus and putamen. In the putamen, treatment-responsive subjects also had decreases in this measure compared to treatment-resistant subjects (34%) (Somerville et al., 2011b). These results provide further support for a biological distinction between treatment response and treatment resistance in schizophrenia. Because treatment-resistant subjects had normal levels of mitochondria per synapse, but treatment responders had fewer mitochondria per synapse than controls, fewer mitochondria per synapse may be related to treatment response.

### 2.2.4. Summary of dorsal striatum.

Fewer mitochondria per synapse were observed in a combined cohort of subjects and does not appear to be caused by APDs. This change was confined to treatment responders, and was not observed in treatment resistant subjects. A decrease in mitochondrial density in the neuropil distinguishes the paranoid from the undifferentiated schizophrenia subgroup. Fewer mitochondria may contribute to the pathophysiology of the illness, may be a medication effect, or an adaptive response to normalize overactive neurotransmission that may occur from the higher than normal number of excitatory striatal synapses previously found (Roberts et al., 2005a, 2005b, 2008, 2012).

### 2.3. Nucleus accumbens

Increases in COX and succinate dehydrogenase have been reported in the nucleus accumbens in schizophrenia postmortem tissue (Prince et al., 1999). However, the structural integrity and general appearance of mitochondria were normal in the schizophrenia group (McCollum et al., 2015). The density of mitochondria in the neuropil, the average diameter, and the number of calcium deposits per mitochondrion were similar between controls and schizophrenia in both the core and shell. Taken together, alterations in mitochondrial function may not be detected with morphology.

### 2.4. Substantia nigra

In spite of the fact that the substantia nigra (SN) and ventral tegmental area (VTA) house the largest proportion of dopamine neurons in the brain and that antipsychotic medication works by blocking dopamine receptors, there have been very few studies of the SN/VTA in schizophrenia.

### 2.4.1. The mitochondrial common deletion

Decrease in the global brain mtDNA common deletion levels are seen in several brain regions in schizophrenia, with the largest abnormalities in dopaminergic regions including the ventral midbrain (Mamdani et al., 2014). The common deletion contains genes encoding subunits of cytochrome oxidase, NADH dehydrogenase and ATP synthase (Samuels et al., 2004; Verge et al., 2011). This abnormality suggests that mitochondrial function is impaired in dopaminergic nuclei including the SN.

### 2.4.2. Cytochrome oxidase

In a recent study COX activity and the protein expression of key subunits for its assembly and activity were measured in postmortem SN/VTA (Rice et al., 2014). While overall COX activity was similar between schizophrenia and NCs, there were decreases in the expression of subunits II and IV-I of COX in schizophrenia in samples containing rostral regions of the SN/VTA. Rats chronically treated with antipsychotic drugs did not show any changes in COX subunit expression, suggesting that the changes seen in the schizophrenia group were not caused by medication. Interestingly, anomalies in COX subunit II mRNA expression have been previously reported in the frontal cortex in schizophrenia without significant changes in COX activity (Clark et al., 1999).

Subunits II and IV of the COX enzyme are crucial for the proper functioning of the COX complex as a whole (Nijtmans et al., 1998; Clark et al., 1999; Rahman et al., 1999). COX subunit II is responsible for the binding of cytochrome c and the subsequent electron transfer to subunit I of the COX enzyme (Taanman, 1997). Suppression of subunit IV has been linked to a reduced function in overall COX activity and an increased susceptibility to apoptosis (Huttemann et al., 2001; Li et al., 2006). These findings suggest that COX subunit expression may be compromised in specific sub-regions of the SN/VTA (i.e. rostral regions), which may lead to a faulty assembly of the enzyme and a greater vulnerability to metabolic insult.

### 2.4.3. Ultrastructure

At the ultrastructural level, mitochondrial hyperplasia has been observed within axon terminals that synapse onto dopamine neurons in the SN (Kolomeets and Uranova, 1999). However, mitochondria in dopamine neuronal somata were similar in size and structural integrity between SZ and NCs (Walker and Roberts, 2016).

## 3. Antipsychotic drugs and mitochondria

The general consensus is that antipsychotic drugs can alter mitochondrial function, number and size. Antipsychotic drugs have differential effects on mitochondrial structure and function depending on brain location, type of antipsychotic drug, length of use, length of withdrawal period, dose and route of administration (for some examples see Takeichi and Sato, 1987; Uranova et al., 1991; Roberts et al., 1995; Prince et al., 1999; Streck et al., 2007). For instance, there are more striatal mitochondria after 3 weeks of haloperidol treatment (Uranova et al., 1991), but less after 6 months (Roberts et al., 1995).

Complexes I, II/III and IV of the electron transport chain can be measured to assess mitochondrial function (Wong-Riley, 1989). Results on the functionality of the various complexes of the electron transport chain in SZ have been conflicting and are beyond the scope of this review. The majority of evidence is in agreement that complex I and succinate dehydrogenase appear to be adversely affected by antipsychotic drugs (Burkhardt et al., 1993; Balijepalli et al., 1999, 2001; Karry et al., 2004; Rosenfeld et al., 2011; Maurer and Moller, 1997; Prince et al., 1997; Streck et al., 2007).

## 4. Summary

In the brains of subjects with schizophrenia, mitochondria are differentially affected depending on the brain region, cell type, and



subcellular location in which they are located (Fig. 3). Moreover, mitochondrial anomalies differ depending on treatment status, treatment response and symptoms. While certain morphological configurations definitely correspond to energy capacity and other functions, it appears that mitochondria can appear intact, while being functionally compromised. Decreases in functional measures may be reflected by decreased number of mitochondria rather than decreased size or structural configuration.

Fewer mitochondria may be a primary deficit of the disease, or mitochondria may die as an epiphenomenon of the disease. Alternatively, mitochondria may be sequestered in neuronal somata located either extrinsic or intrinsic to the striatum. An inability of mitochondria to move into axon terminals or dendrites could account for a decreased number of mitochondria in these structures. Since mitochondria move around the neuron along microtubules between the soma and processes, damage to cytoskeletal elements may lead to a failure of proper mitochondrial movement. In the cingulum bundle, our preliminary work shows abnormal levels of  $\alpha$ tubulin, a component of microtubules, in off drug schizophrenia, which is normalized by APD treatment (Roberts and Schoonover, 2016). This suggests cytoskeletal abnormalities.

Numerous studies have reported the occurrence of altered or decreased mitochondrial function in the aging brain, in neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's disease, in neurometabolic disorders such as Leigh syndrome (Dahl, 1998) and in psychiatric disorders other than schizophrenia (Orth and Schapira, 2001; Vila et al., 2008; Rollins et al., 2009; Anglin et al., 2012; Manji et al., 2012; Ottolini et al., 2013). Thus, it is clear that mitochondria play a fundamental role in many brain disease processes.

#### Conflict of interest

The author has no conflicts of interest to declare.

#### Contributors

Dr. Roberts wrote this review based in part on previously published work generated in her laboratory.

#### Role of funding sources

This research was supported by grants RO1MH66123 and R21MH108867-01 from the National Institute of Mental Health. The funding source had no role in study design, data collection, analysis and interpretation of data, writing the manuscript, or in the decision to submit the paper for publication.

#### Acknowledgments

I would like to thank the past and present members of my lab whose work I cite in this review, especially Keri A. Barksdale, Miguel Melendez-Ferro, Lesley A. McCollum, Emma Perez-Costas, Matthew W. Rice, Joy K. Roche, Shahza M. Somerville, Kirsten E. Schoonover, and Courtney K. Walker.

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