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Substantia nigra ultrastructural pathology in schizophrenia

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ABSTRACT

Schizophrenia is a severe mental illness affecting approximately 1% of the population worldwide. Despite its prevalence, the cause remains unknown, and treatment is not effective in all patients. Dopamine is thought to play a role in schizophrenia pathology, yet the substantia nigra (SN), the origin of dopaminergic pathways, has not been studied extensively in schizophrenia. In this study, electron microscopy was used to examine neurons, oligodendrocytes, and myelinated axons in the SN of normal controls (NCs, n = 9) and schizophrenia subjects with varying response to antipsychotic drugs [SZ, n = 14; treatment resistant (TR) = 6, treatment responsive (RESP) = 6, unknown = 2]. Postmortem tissue was analyzed for qualitative and quantitative markers of ultrastructural integrity. A significantly higher percentage of axons in the schizophrenia group had inclusions in the myelin sheath compared to NCs (SZ: 3.9 ± 1.7, NC: 2.6 ± 2.0). When considering treatment response, a significantly higher percentage of axons lacked cytoplasm (TR: 9.7 ± 5.5, NC: 3.5 ± 2.3), contained cellular debris (TR: 7.5 ± 3.2, NC: 2.3 ± 1.3) or had protrusions in the myelin sheath (TR: 0.4 ± 0.5, NC: 0.2 ± 0.3). The G-ratio, a measure of myelin thickness, was significantly different between treatment response groups and was greater in TR (0.72 ± 0.02) as compared to NCs (0.68 ± 0.03), indicating decreased myelination in TR. These findings, which suggest myelin pathology in the SN in schizophrenia, are consistent with findings elsewhere in the brain. In addition, our results suggest cytoskeletal abnormalities, which may or may not be associated with myelin pathology.

1. Introduction

Schizophrenia (SZ), a severe mental illness affecting approximately 1% of the population, is characterized by positive, negative, and cognitive symptoms (American Psychiatric Association, 2013). Antipsychotic drugs (APDs), used to treat SZ (see as review Seeman, 2002), function by blocking striatal dopamine D_2 receptors (Carlsson and Lindqvist, 1963; Creese et al., 1977). The substantia nigra (SN) and ventral tegmental area are the major origin of dopaminergic pathways (Fallon and Moore, 1978; Fallon et al., 1978; Gaspar et al., 1992). While many studies have examined targets of the SN in SZ, far fewer studies have examined the SN itself, and these have often reported conflicting results.

Imaging studies have shown that more dopamine is produced in the SN in SZ (Watanabe et al., 2014), there is evidence of higher glutamate levels (White et al., 2015), and the SN is hyperactive, which is linked with both prefrontal cortex hypofunction and striatal hyperfunction (Yoon et al., 2013, 2014). Some postmortem studies show complementary evidence, such as increased levels of tyrosine hydroxylase (TH) mRNA or protein, indicating elevated dopamine synthesis capacity (Mueller et al., 2004; Toru et al., 1988; Howes et al., 2013; Schoonover et al., 2017). However, some have found no difference (Ichinose et al., 1994) or decreased levels of TH protein in rostral SN (Perez-Costas et al., 2012). The deficit of TH protein rostrally was due to decreased TH translation, rather than transcription (Perez-Costas et al., 2012) or neuronal loss (Rice et al., 2016). Additionally, abnormal expression of key cytochrome *c* oxidase subunits was seen in the SN in SZ (Rice et al., 2014), suggesting metabolic abnormalities.

Abnormalities in oligodendrocytes and myelinated axons have been observed at imaging, genetic, and ultrastructural levels. Decreased white matter volume has been seen in SZ (Bora et al., 2011; De Peri et al., 2012; Di et al., 2009; Haijma et al., 2013; Kubicki et al., 2007; Kuswanto et al., 2012; Olabi et al., 2011; Samartzis et al., 2014; Yao et al., 2013), suggesting oligodendrocyte and myelin pathology. Several genes associated with oligodendrocyte function and the myelin sheath are downregulated in SZ, altering their development and function (Xiao et al., 2008). Ultrastructural studies have shown oligodendrocyte pathologies, including swollen appearance, chromatin condensation, and membranous myelin-like inclusions, as well as altered myelin thickness and a greater percentage of pathologically myelinated fibers in prefrontal cortex (Uranova et al., 2011; Vikhreva et al., 2016).

Despite the prevalence of SZ, few advances have been made in the refinement of APDs. Generally, APDs alleviate positive symptoms with little to no effect on negative symptoms and cognitive impairment (McEvoy, 2006). Furthermore, treatment response is not uniform

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among patients. One-fifth to one-third of patients do not respond to APDs, while the remainder show varying levels of recovery (Conley and Kelly, 2001). Although what causes these differences in response is unknown, there is evidence of a biological basis (Altamura et al., 2005; Arango et al., 2003; Beerpoot et al., 1996; Roberts et al., 2012; Sheitman and Lieberman, 1998; Somerville et al., 2011b).

The goal of this study was to determine whether there are structural differences in the SN that are associated with SZ pathology and treatment response. Electron microscopy of postmortem human tissue was used to analyze neurons, oligodendrocytes, and myelinated axons. Because animal models of SZ do not perfectly replicate the disease, postmortem studies such as this one are valuable in SZ research.

2. Methods

2.1. Tissue samples and preparation

Postmortem human brain tissue was obtained from the Maryland and Alabama Brain Collections from 9 controls and 14 schizophrenia subjects with permission of next of kin. Cases were diagnosed by two psychiatrists' independent evaluations, as described previously (McCollum et al., 2015). Diagnosis of treatment response or resistance was conducted as detailed by Roberts et al. (2009, 2012). Briefly, treatment response or resistance was diagnosed based on established criteria (Conley and Kelly, 2001; Kane et al., 1988: (1) no clinical improvement during two prior drug treatment periods; (2) at least five years with no period of good social or occupational functioning; and (3) presence of persistent positive psychotic symptoms throughout the person's life. If all were present, a diagnosis of treatment resistance was made. Control and schizophrenia subjects were matched for age, race, gender, pH, and postmortem interval, as shown in Table 1.

The midbrains, containing the SN, were immersed in a cold solution (4 °C) of 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer (PB). For most of the cases used in the present study, the dorsal striatum (see for examples Roberts et al., 2009, 2012; Somerville et al., 2011a, 2011b), nucleus accumbens (McCollum et al., 2015), and prefrontal cortex (see for example Roberts et al., 2005) have been previously analyzed for synaptic organization. The tissue was stored at 4 °C in fixative until used. The SN was blocked in the transverse plane at the level of the 3rd nerve rootlets and red nucleus. Six series of 40 μm sections were cut using a Vibratome (HM 650V microtome, Thermo Scientific).

2.2. Electron microscopy

Samples were flat-embedded for electron microscopy using standard techniques, described by McCollum and Roberts (2014). Briefly, the sections were rinsed twice in PB for 5 min each, immersed in 1% osmium tetroxide in 0.1 M PB at room temperature in the dark for 1 h, rinsed four times for 5 min each in PB, then dehydrated at room temperature in the dark in 50% and then 70% EtOH. The tissue was stained en bloc in a 1% uranyl acetate solution in 70% EtOH for 1 h for contrast, and then rinsed in 70% EtOH two times for 5 min each. The tissue was dehydrated in increasing concentrations of EtOH, followed by 100% propylene oxide, then embedded in epon resins, and heated at 60 °C for 72 h.

For each case, blocks at least 240 μm apart rostrocaudally, were used to obtain semithin sections. The location of these blocks was in the dorsal tier of the substantia nigra pars compacta in the middle of the mediolateral SN. These sections (250 nm thickness) were collected using an ultramicrotome (Leica EM UC6), mounted on glass slides, stained with Toluidine Blue and cover slipped for reference. These sections were used to verify that the block face contained dopaminergic neurons (identified by size and neuromelanin pigment). Serial thin sections (90 nm thickness) were collected, mounted on Formvar-coated copper grids, and photographed at 80KV on a Hitachi 87650 transmission electron microscope using a Hamamatsu ORCA-HR digital camera.

Micrographs were taken at the following magnifications: neurons, 5,000X; myelinated axons and oligodendrocytes, 10,000X; and neuronal rough endoplasmic reticulum (rER), 25,000X. A total of 230 neurons, 313 oligodendrocytes, and 5336 myelinated axons were photographed and analyzed. On average, 26 micrographs of rER were photographed per case. Authors were blinded to case diagnosis throughout microscopy and data collection.

2.3. Data collection

2.3.1. Neurons and rER

At least ten dopamine neurons, identified by morphological criteria, were photographed and analyzed per case. Dopaminergic neurons were identified by their large cell bodies that usually contained neuromelanin granules and numerous stacks of rER as defined by Domesick et al. (1983) (Fig. 1A,B). Neurons too large to fit in one micrograph were stitched together using PanaVue ImageAssembler3. Data were obtained from single thin sections. Mitochondria (n = 13,458) in the somata were numbered in Adobe Photoshop C4 and diameters were measured along the short axis using ImageJ. The number of mitochondria per square micron of cytoplasm was calculated using ImageJ.

Cells contained variable amounts of rER. If a neuron contained similar rER throughout, approximately 3 micrographs were taken. In those containing clusters with notably different organization, micrographs were taken of the different clusters to ensure the overall rER organization was well-represented. rER organization was assessed using a rating scale, that ranged from 1 to 4: 1, highly disorganized and mostly scattered ribosomes; 2, a few strands; 3, at least 3 parallel strands; and 4, four or more perfectly organized strands (Fig. 1C–F). Averages of rER organization ratings were calculated per case.

2.3.2. Oligodendrocytes

At least 10 oligodendrocytes, identified by morphological criteria, were photographed per case. Oligodendrocytes were identified by a round or oval-shaped nucleus with heterochromatin on the nuclear border (Uranova et al., 2001), light to medium density cytoplasm and defined cellular borders (Fig. 2). Areas of oligodendrocyte soma, nuclei, heterochromatin, and euchromatin were measured, and the percent

Table 1
Case demographics.

	#	Age	pH	PMI (h)	Age of onset	Duration	APD		Race	Sex
NC	9	43.9 ± 14.3	6.9 ± 0.3	5.9 ± 1.5					4AA, 5C	4F, 5M
SZ	14	48.3 ± 11.4	6.9 ± 0.3	6.1 ± 2.3	22.3 ± 5.6	24.8 ± 10.5	7A, 4T		7AA, 7C	5F, 9M
t-test		0.42	0.85	0.84				χ^2	0.80	0.68
TR	6	40.2 ± 8.4	6.8 ± 0.2	7.0 ± 2.8	19.8 ± 4.7	24.3 ± 8.9	4A, 0 T		2AA, 4C	3F, 3M
RESP	6	51.3 ± 8.8	7.0 ± 0.3	5.2 ± 1.9	24.4 ± 5.8	25.2 ± 12.6	2A, 4 T		3AA, 3C	2F, 4 M
ANOVA/*t*-test		0.25	0.82	0.32	0.24	0.90	**0.007**	χ^2	0.56	0.56

Mean ± standard deviation, NC = normal control, SZ = schizophrenia, RESP = treatment responder, TR = treatment resistant, AA = African American, C = Caucasian, F = female, M = male, PMI = postmortem interval.

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