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Decreased synaptic and mitochondrial density in the postmortem anterior cingulate cortex in schizophrenia

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ABSTRACT

Schizophrenia (SZ) is a mental illness characterized by psychosis, negative symptoms, and cognitive deficits. The anterior cingulate cortex (ACC), a structurally and functionally diverse region, is one of several brain regions that is abnormal in SZ. The present study compared synaptic organization and mitochondrial number and morphology in postmortem ACC in SZ versus normal control (NC). Total synaptic density in the combined ACC was decreased in SZ, to 72% of normal controls (NCs), due to selective decreases in axospinous synapses, both asymmetric (excitatory) and symmetric (inhibitory). These changes were present in layers 3 and 5/6. The density of mitochondria in all axon terminals combined in SZ was decreased to 64% of NC. In layer 3, mitochondrial density was decreased only in terminals forming asymmetric synapses with spines, while in layers 5/6 mitochondrial density was decreased in terminals forming symmetric synapses with spines and dendrites. The proportion of terminals making symmetric synapses that contained mitochondria was significantly lower in SZ than in NCs, especially for symmetric axospinous synapses. The number of mitochondria per neuronal somata was decreased in the ACC in SZ compared to NCs; this finding was present in layers 5–6. The size of mitochondria in neuronal somata and throughout the neuropil was similar in SZ and NCs. Our results, though preliminary, are well supported by the literature, and support an anatomical substrate for some of the altered executive functions found in SZ.

1. Introduction

The anterior cingulate cortex (ACC) is part of prefrontal cortex and its neuroanatomy has been well described (DeFelipe et al., 2002; Jones, 1998; Lewis et al., 2002; Peters, 2002). The ACC is composed of distinct anatomical subregions, each with different functional properties (Peterson et al., 1999; Palomero-Gallagher et al., 2008; Vogt et al., 1992). At the microscopic level, each layer of the ACC has characteristic types of neurons, which project to particular targets; each layer is the recipient of inputs from specific regions (DeFelipe et al., 2002; Lewis et al., 2002). Human imaging studies indicate that the dorsal ACC is involved in mediating attention and executive functions, such as task difficulty, remote memory (Koski and Petrides, 2001), conflict (Barch et al., 2001; Kerns et al., 2004), response inhibition and error commission (Braver et al., 2001; Mathalon et al., 2002). The subcallosal ACC is involved in emotional processing (Bush et al., 2000) or internal states (Greicius et al., 2003), the dorsal ACC is involved in cognitive function, and the rostral ACC, located between these two subdivisions, plays an important role in the integration of these functions (Vogt et al., 1992).

The ACC is one of several brain regions that are abnormal in schizophrenia (SZ), as shown in both *in vivo* imaging and postmortem studies (Fornito et al., 2009). *In vivo* imaging of people with SZ has shown abnormalities in multiple transmitter systems (Egerton et al., 2012; Kraguljac et al., 2012; Rowland et al., 2013; Théberge et al., 2003), blood flow (Holcomb et al, 2000) and metabolism (Nordahl et al., 1996; Tamminga et al., 1992). Functional impairments in cognitive interference (Heckers et al., 2004), error or conflict monitoring (Alain et al., 2002; Carter et al., 1997, 2001) and response monitoring (Kopp and Rist, 1999; Mathalon et al., 2002) have also been demonstrated.

Postmortem studies have shown multiple defects in the ACC in SZ (Eastwood and Harrison, 2001; Fornito et al., 2009). Neurochemical and molecular changes include altered distribution or modulation of dopamine (Benes et al., 1997), abnormalities in multiple aspects of the glutamate system (Barksdale et al., 2014; Bauer et al., 2008, 2010; Drummond et al., 2013; Katsel et al., 2011; Oni-Orisan et al., 2008; Woo et al., 2004, 2007) and intracellular signaling abnormalities (Funk et al., 2012, 2014). Mitochondrial pathology has been implicated repeatedly in schizophrenia (Anglin et al., 2012; Manji et al., 2012) and in the ACC, oxidative stress, a result of mitochondrial production of reactive oxygen species, is increased (Wang et al., 2009). Anatomical abnormalities include layer specific alterations in neuronal distribution and density (Benes and Bird, 1987; Brune et al., 2010; Todtenkopf et al., 2005), and increased

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numbers of glutamatergic (Benes et al., 1987, 1992) and parvalbumin axons (Kalus et al., 1997, 1999). Of note, previous electron microscopic studies in SZ have shown layer specific abnormalities such as fewer synapses in the ACC (Aganova and Uranova, 1992) and alterations in synapses, mitochondria and oligodendrocytes in other areas of prefrontal cortex (Uranova et al., 2004, 2007, 2011).

The purpose of the present study is to compare the synaptic organization and mitochondrial number and morphology in SZ versus normal control postmortem ACC. Several synaptic features were quantified, including morphological features which identify excitatory and inhibitory synapses. This work has been presented in preliminary form (Barksdale et al., 2012a,b; Roberts et al., 2013).

2. Methods

Human brain tissue was obtained with IRB approved protocols from the Maryland Brain Collection and the Alabama Brain Collection. Diagnostic criteria have been described previously (McCollum et al., in press; Roberts et al., 2008). Demographics are presented in Table 1.

Coronal blocks from the dorsal ACC were preserved in fixative and processed for electron microscopy as previously described (McCollum et al., in press). One series of sections was stained for Kluver-Barrera stain as previously described (Bolding et al., 2013; Roberts et al., 2014) and used to identify the layers of the cortex (Fig. 1).

A series of tissue adjacent to the ones used for Kluver-Barrera was processed for electron microscopic analysis using standard techniques (McCollum et al., in press; Roberts et al., 2008). Two to three samples per case, at least 240 μm apart, were used for quantitative analysis. Each sample was cut into an average of 10–11 serial ultrathin sections (90 nm thickness), mounted on Formvar-coated copper grids, and photographed at 80 kV on a Hitachi transmission electron microscope. To determine the number of synapses in the neuropil and mitochondria in terminals, these serial sections were analyzed using the physical disector technique (Geinisman et al., 1996; Perez-Costas et al., 2007) from layers 3 and 5/6 (Fig. 2). In each section, eight pictures were photographed at a magnification of 15,000 and stitched together to form a montage. The images are enlarged by 50% for synapse and mitochondria counting. These methods for identifying and quantifying profiles have been described in detail by us (Roberts et al., 2008; Somerville et al., 2011, 2012). Briefly, neuropil only was quantified; cell bodies were not photographed. For controls (combined layer 3 and layers 5/6), a total of 2313 synapses were counted in a total volume of 11,559 μm^3; the average per case was 386 synapses in a volume of 1926 μm^3. For SZ cases (combined layer 3 and layers 5/6), a total of 1309 synapses were counted in a total volume of 9248 μm^3; the average per case was 327 synapses in a volume of 2320 μm^3. A total of 712 and 356 mitochondria were identified in axon terminals in NC and SZ, respectively.

In addition, mitochondria throughout the neuropil in 1–2 randomly selected montages per case per layer were counted and their diameters were measured. For this analysis, an average of 1470 um^2 per case was analyzed and an average of 289 mitochondria per case were counted and measured. Also, neuronal somata near the center of the neuron were photographed at 5000–8000 magnification and the number of mitochondria present in a single section of each soma was counted. For this analysis a total number of 174 neurons containing a total of 2881 mitochondria were counted and their diameters measured.

The individuals counting the synapses and mitochondria and measuring mitochondria were blinded to the identity of the groups. The results were compared with unpaired t-tests.

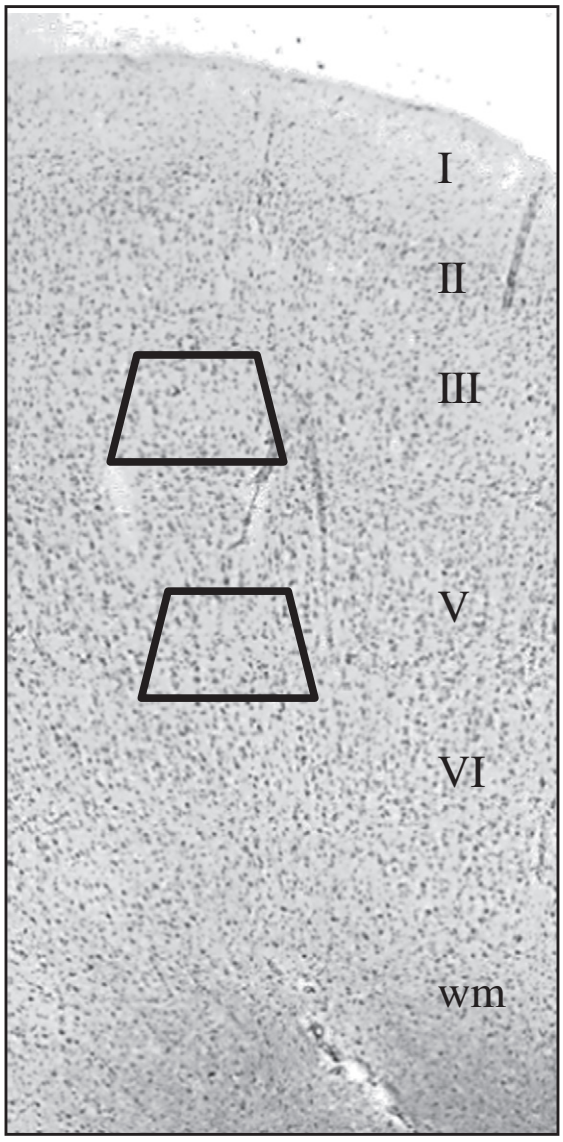

Fig. 1. Kluver-Barrera stained section of dorsal ACC. Cortical layers and subcortical white matter (wm) are indicated. Trapezoids show the typical locations of samples taken from layers 3 and 5/6.

Table 1
Demographic information for the cases. NA, information not available; NCs, normal controls; ARS, age, race, sex; PMI, postmortem interval in hours; C, Caucasian; AA, African American; M, male; F, female; and P values indicate the results of independent t-tests or Pearson Chi-Square tests between groups.

NCs	Source	ARS	PMI	Nicotine	Alcoholism	P values
1	MBC	32CF	7.0	Yes	No	Age: 0.311
2	MBC	45AAF	6.75	NA	Yes	Race: 0.598
3	MBC	40AAM	7.0	Yes	No	Sex: 1.000
4	ABC	87CM	7.5	No	No	PMI: 0.425
5	ABC	32CF	7.0	Yes	No	Nicotine: 0.459
6	ABC	61CM	4.0	Yes	No	Alcoholism: 0.747
Mean		49.5	6.54			
SD		21.3	1.27			
SZ	source	ARS	PMI	Nicotine	EtOH	Medication
1	MBC	60AAM	5.0	NA	No	Olanzapine
2	MBC	60AAF	6.0	NA	No	Typical
3	ABC	56CM	7.5	Yes	Yes	Olanzapine Quetiapine
4	ABC	70CF	5.0	Yes	No	Depakote; Abilify
Mean		61.5	5.88			
SD		6.0	1.18			