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Neurobiology of Disease

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Neurobiology of Disease 20 (2005) 324 – 335

Synaptic differences in the patch matrix compartments of subjects with schizophrenia: A postmortem ultrastructural study of the striatum

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Received 14 September 2004; revised 9 February 2005; accepted 21 March 2005
Available online 26 April 2005

The striatum processes motor, cognitive, and limbic circuitry. Striatal patch and matrix compartments are organized differently in many aspects including connectivity. Abnormalities in either compartment could have different functional consequences. The present study compares the synaptic organization in the patches and matrix in subjects with schizophrenia (SZ, $n = 14$) versus normal controls (NC, $n = 8$). Postmortem striatal tissue was processed for calbindin immunocytochemistry to identify the patch versus matrix compartments, prepared for electron microscopy, and analyzed using stereology. Several synaptic changes were observed in the SZ subjects vs. NCs including a higher density of cortical-type synapses in the putamen patch (44% higher) and in the caudate matrix (36% higher) in SZ cases on typical antipsychotic drugs. These changes appeared to be normalized rather than caused by treatment. The abnormal connectivity may represent a failure of normal synaptic pruning and may play a role in limbic or cognitive dysfunction in schizophrenia.

Keywords: Schizophrenia; Striatum; Postmortem

Introduction

The caudate nucleus and putamen receive cortical inputs from motor, premotor, dorsolateral prefrontal, orbital and medial prefrontal, and anterior cingulate (Alexander et al., 1986; Chikama et al., 1997; Eblen and Graybiel, 1995; Goldman-Rakic, 1994; Goldman-Rakic and Selemon, 1986; Haber et al., 1995, 2000; Kunishio and Haber, 1994; Parent and Hazrati, 1995; Smith et al., 1998). Motor, cognitive, and limbic functions modulated by the striatum are all are abnormal in schizophrenia (Harrison, 1999; Powers, 1999). In vivo studies show that the striatum in subjects with schizophrenia displays augmentation of presynaptic dopamine function, indicating an increase in dopamine synthesis capacity and an increase in presynaptic dopamine stores (Abi-Dargham et al., 1998, 2000; Breier et al., 1997; Dao-Castellana et al., 1997; Hietala et al., 1994, 1995, 1999; Laruelle et al., 1996, 1999). Numerous studies have shown that striatal metabolic rate is diminished in people with schizophrenia (Bartlett et al., 1991; Buchsbaum et al., 1987, 1992a,b; Cleghorn et al., 1992; DeLisi et al., 1985; Resnick et al., 1988). In contrast, formal thought disorder in schizophrenic subjects is positively correlated with activity in the caudate (Kircher et al., 2001; McGuire et al., 1998). There is also evidence of mitochondrial malfunction in the striatum of subjects with schizophrenia (Ben-Shachar, 2002; Kung and Roberts, 1999; Maurer et al., 2001; Prince et al., 1999).

Postmortem studies indicate anatomical, neurochemical, and receptor abnormalities as well (Holt et al., 1999; Kleinman et al., 1985; Korpi et al., 1986; Lahti et al., 1998; Simpson et al., 1992). Electron microscopic studies of the striatum in subjects with schizophrenia have shown smaller spine size, fewer mitochondria, and other evidence of pathology in neurons and glia (Kung and Roberts, 1999; Kung et al., 1998a; Roberts et al., 1996; Uranova et al., 1996, 2001). In previous studies from our laboratory, synaptic abnormalities were found including an increase in asymmetric axospinous synapses typical of cortical striatal inputs (Kung et al., 1998a; Roberts et al., 2001). The morphological characteristics of synapses such as the symmetry of the postsynaptic density and the type of postsynaptic target provide information on the putative origin of the neurons forming the synapses and whether the neural transmission may be excitatory or inhibitory. The majority of synapses present in the human striatum are asymmetric (Roberts and Knickman, 2002; Roberts et al., 1996), characteristic of excitatory synaptic transmission. In experimental animals, the majority of terminals forming asymmetric axospinous synapses originate from neurons in the cortex (Kemp and Powell, 1971a,b,c), with less extensive inputs arising from the thalamus (Chung et al., 1977; Kemp and Powell, 1971a,b,c; Sadikot et al., 1992a,b; Smith et al., 1994) and the raphe (Lavoie and Parent, 1990; Pasik and Pasik, 1982). Asymmetric axodendritic synapses arise from the thalamus (Sadikot et al., 1992a,b; Smith et al.,

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Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2005.03.015

1994), the amygdala (Kita and Kitai, 1990), and the raphe (Pasik and Pasik, 1982). Symmetric synapses, typical of inhibitory transmission, originate from several sources including striatal neurons (DiFiglia, 1987; DiFiglia and Aronin, 1982; Kitai et al., 1979; Ribak et al., 1979; Somogyi et al., 1981; Wilson and Groves, 1980) and dopamine neurons (Freund et al., 1984; Kubota et al., 1987a,b; Kung et al., 1998b; Pickel et al., 1981).

We were interested in extending our studies of synaptic organization in the striatum to include an analysis of the patch (striosome) and matrix compartments. These two anatomically distinct striatal compartments (Graybiel and Ragsdale, 1978) differ from each other in several ways including neurochemical composition (Graybiel and Ragsdale, 1978; Holt et al., 1997; Prensa et al., 1999), neuronal organization (Hirsch et al., 1989; Holt et al., 1997; Kubota and Kawaguchi, 1993; Penny et al., 1988), developmental schedule (Graybiel and Hickey, 1982; Liu and Graybiel, 1992; Van der Kooy and Fishell, 1987), behavioral function (White and Hiroi, 1998), connectivity (Gerfen, 1989, 1992; Gerfen et al., 1985; Kincaid and Wilson, 1996; Ragsdale and Graybiel, 1991), and response to antipsychotic drugs (Bubser and Deutch, 2002). Importantly, the patches and matrix have distinct afferent and efferent connections and changes in the connectivity within the patch vs. matrix compartments could have different downstream effects (Gerfen, 1987, 1989; Gerfen et al., 1985; Holt et al., 1997). In addition to the anatomical heterogeneity observed at the light microscopic level between the patches and the matrix, these compartments are also heterogeneous at the ultrastructural level in humans (Roberts and Knickman, 2002). Notably, there is a differential density of synapses, characteristic of cortical inputs, in the patches vs. the matrix in control human striatum (Roberts and Knickman, 2002). Interestingly, the synaptic density in the putamen patches is similar to that of the caudate matrix, whereas, the synaptic density of the putamen matrix is similar to that of the caudate patches. The purpose of the present study was to compare the synaptic organization in the patch and matrix compartments of the normal control postmortem caudate and putamen to that of subjects with schizophrenia. The results will provide a more detailed picture of where the anatomical abnormalities occur in the striatum of subjects with schizophrenia. Thus, we will test two hypotheses: that there is a differential degree of synaptic density changes in the patches vs. the matrix and that this abnormality is not related to antipsychotic drug use. This work has been published in preliminary form (Roberts et al., 2001, 2003).

Methods

Subjects

Postmortem human brain tissue was obtained with family permission from the Maryland Brain Collection within 8 h of death from eight control cases and fourteen cases with schizophrenia (SZ). Controls were matched to the SZs using age, sex, postmortem interval (PMI), and race (when possible) as criteria. Information on the cases was collected from autopsy reports, medical records, and family interviews. Controls were defined as people without a lifetime diagnosis of a serious mental illness, other than alcohol abuse or dependence. According to DSM-IV criteria, diagnosis of schizophrenia was made independently by two research psychiatrists using the SCID (Scheduled Clinical Interview for the DSM-IV) (Spitzer et al., 1992) and the Diagnosis Evaluation After Death (DEAD) (Salzman et al., 1983). Medication status was verified for the period of 1, 3, and 6 months prior to death. Risperidone, a second-generation antipsychotic, was considered to be an atypical antipsychotic, based on the dosage and the fact that the cases treated with this drug had chronic schizophrenia (Love et al., 1999; Kapur et al., 1999; Remington and Kapur, 2000); the response of neuroleptic naïve patients to risperidone is more similar to that of a typical APD (Rosebush and Mazurek, 1999). Demographics and diagnoses for all cases are shown in Table 1. The University of Maryland Institutional Review Board approved all procedures.

Immunocytochemistry

Coronal blocks (1 cm thick) of the striatum were immersed in a cold solution of 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2–7.4, (PB) for a period of at least 1 week. Striatal tissue was cut with a Vibratome (at a thickness of 40 μm) and the sections were collected into cold PB. One series out of six was processed for the immunocytochemical localization of calbindin D28K (Sigma) to identify calbindin-poor striosomes (patches) and calbindin-rich matrix. Free-floating sections (240 μm apart) were washed in PB (3 × 10 min), incubated in 2% normal serum in PB for 30 min, and followed by the primary antibody at a dilution of 1:20,000 for 60 h. The tissue was then treated with reagents from the avidin–biotin peroxidase kit (ABC standard kit) using recommended dilutions and times (Hsu et al., 1981). The sections were washed in PB (3 × 10 min), incubated for 60 min in biotinylated horse anti-mouse IgG (44 μl/10 ml PB), washed, incubated for 30 min in the avidin–biotin complex (88 μl each of solutions A and B/10 ml PB) and washed. Sections were then incubated in diaminobenzidine (6 mg/10 ml PB) containing 0.03% hydrogen peroxide for 5 to 30 min to visualize the reaction product. Controls consisted of eliminating the primary antibody but otherwise processing the tissue in an identical fashion; control sections did not exhibit any specific staining.

Electron microscopy

Tissue samples approximately 0.5 × 1.0 cm in size were cut from the caudate and putamen from each case. From each case, pieces of the caudate and putamen were embedded in separate vials, in case of breakage during the embedding process. Briefly, the sections were rinsed in PB (3 × 10 min), immersed in 1% osmium tetroxide for 1 h, stained en bloc with 1% uranyl acetate for 2 h, dehydrated in alcohol, embedded in resins, flat embedded and heated at 60°C for 72 h. Samples were randomly chosen from both the caudate and putamen from the patch or matrix compartments (as identified by low or high calbindin staining, respectively) as previously described (Roberts and Knickman, 2002). The samples were cut out, and mounted on beem capsules. Using a glass knife, several semi-thin 1- to 2-μm sections were cut from the face of the block to remove most of the immunoreactivity. Note that the calbindin immunolabeling was used to identify the patch vs. the matrix compartments, but was not necessary for the ultrastructural analysis. The blocks were then thin sectioned at a thickness of 90 nm. The average length of each ribbon was 14 ± 3.3 serial sections.

Data collection and synapse counting

Quantitative analysis was performed from the head of the caudate and anterior putamen. Regions of the neuropil (excluding