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Ultrastructural Distinctions Between Treatment Responders and Non-Responders in Schizophrenia: Postmortem Studies of the Striatum

Rosalinda C. Roberts[1], Joy K. Roche[1],
Shahza M. Somerville[2] and Robert R. Conley[3]
[1]Department of Psychiatry and Behavioral Neurobiology
University of Alabama, Birmingham, Birmingham, AL
[2]Technical Resources International, Bethesda, MD
[3]Department of Neuroscience , Lilly Technology Center, Indianapolis, IN
USA

1. Introduction

1.1 Schizophrenia

Schizophrenia (SZ) typically manifests itself in early adulthood with psychotic symptoms (hallucinations, delusions, disorders of thought or speech, grossly disorganized behaviour), cognitive impairments and in some, negative symptoms. This illness affects 1% of the population worldwide (APA, 1994). Risk factors for schizophrenia suggest both a developmental and genetic basis. Neuropathology and abnormalities in multiple neurotransmitter systems have been reported throughout the brain (Harrison 1999; Powers 1999). However, there is no diagnostic pathology that identifies the brains of SZ subjects.

1.2 Treatment response/resistance

Antipsychotic drugs (APDs) act primarily to relieve positive symptoms with little or no effect on negative (i.e. social withdrawal, anhedonia, avolition) and cognitive symptoms (McEvoy 2006). Not all patients respond to treatment and in those who do, only psychotic symptoms are usually improved (Conley & Kelly 2001; Meltzer 1997). Treatment response to APD is best defined along a gradient, one end of which is characterized by no response (TNR) also referred as "treatment resistant". The reported rate of treatment response can vary from 25 to 70% (Brenner, et al., 1990). The reason for treatment resistance (or nonresponse) is poorly understood but appears to have a biological basis (Altamura et al., 2005; Beerpoot et al., 1996; Sheitman and Lieberman, 1998). A relationship between pathophysiology in SZ and the degree of treatment response has been shown in several neuroimaging studies (Arango et al., 2003; Rodriguez et al., 1997; Staal et al., 2001). MRI studies have shown that treatment nonresponsive SZ subjects have greater cortical atrophy in certain regions (Mitelman et al., 2005), smaller putamen volumes (Mitelman et al., 2009) and larger cerebral ventricles than do treatment responsive SZs (Bilder et al. 1994; Staal et al., 2001; Stern et al., 1993). SPECT shows

differential values for cerebral perfusion, an index of neuronal activity (Gemmell et al., 1990; Turkington et al., 1993), in treatment responsive vs. resistant SZ subjects (Rodriguez et al., 1997). APD naïve SZ subjects who eventually respond to treatment have elevated dopamine release compared to those subjects who do not eventually respond (Abi-Dargham et al. 2000). Importantly, treatment resistance does not occur because of a failure of D_2 receptor blockade by APDs as these treatment resistant subjects show a 95% blockade of striatal D_2 receptors following typical APD treatment (Coppens et al., 1991). Neurobiological differences between treatment response and treatment resistance in SZ are rarely studied at the microscopic level in postmortem tissue, but provide a strategy for trying to link psychosis with particular neuropathology (Roberts et al., 2009; Somerville et al., 2011b). Although numerous neuroimaging studies suggest a biological basis to treatment response/resistance, to our knowledge, only our postmortem studies have addressed this issue (Roberts et al., 2009; Somerville et al., 2011b).

The striatum is rich in dopamine receptors and all known effective APDs block dopamine D_2 receptors (Creese, et al., 1976; Lahti et al., 2003; Seeman et al., 1975). Dopamine modulation depends on many factors such as receptor subtype and location (Cepeda et al., 2001; Onn et al., 2000; West and Grace, 2002), the concentration of ambient dopamine and the activity state of the spiny neuron (Cepeda & Levine, 1998). Brain imaging studies show that the striatum of subjects with SZ displays augmentation of presynaptic dopamine function, indicating an increase in dopamine synthesis capacity and/or an increase in presynaptic dopamine stores (Abi-Dargham et al., 1998, 2000; Breier et al., 1997; Dao-Castellana et al., 1997; Hietala et al., 1995, 1999; Laruelle et al., 1996, 1999). Specifically, there is an increase in the release of dopamine (Abi-Dargham et al., 1998; Laruelle et al., 1996, 1999) and in the density and occupancy of dopamine D_2 receptors (Abi-Dargham et al., 2000; Wong et al., 1986). Patients with SZ with high dopamine release are far more responsive to APDs than those patients who have dopamine levels lower than or comparable to that of healthy volunteers (Abi-Dargham et al., 2000). In addition, dopamine D_2 receptor density in the caudate nucleus is higher in the unaffected monozygotic twins of SZ subjects compared to unaffected dizygotic twins and healthy control twins (Hirvonen et al., 2005). The studies suggest that dopamine transmission dysfunction confers a genetic risk for schizophrenia.

1.3 Striatal pathology in schizophrenia

The striatum of subjects with schizophrenia shows several pathological abnormalities in vivo (Buchsbaum & Hazlett, 1998) and in postmortem tissue (Harrison, 1999; Powers, 1999). Grossly, the striatum of neuroleptic-naïve schizophrenia subjects is smaller than normal, but upon antipsychotic treatment with several but not all drugs, the striatum enlarges (Brandt & Bonelli, 2008; Chakos et al., 1994). Surface deformation mapping results have shown localized volume decreases in both the caudate nucleus and putamen in neuroleptic free patients; such changes were most pronounced in the associative striatum (Mamah et al., 2007). Moreover, affective flattening was correlated with abnormalities in the anterior putamen (Mamah et al., 2007). Also, the unaffected siblings of schizophrenia patients showed intermediate changes between that of controls and their ill siblings (Mamah et al., 2008). Offspring of schizophrenia patients also have smaller caudate nuclei (Rajarethinam, et al., 2007). Taken together, these data suggest that gross morphological changes in the caudate nucleus and/or putamen may be a core feature of the illness or confer a risk factor. Consistent with the imaging data, results from microscopy show a 10% decrease in cell

number in the caudate nucleus and putamen (Kreczmanski et al., 2007). Neurochemical deficits include decreases in 1) uptake sites for glutamate and GABA (Simpson et al., 1992), 2) excitatory amino acid transporter 3 and vesicular glutamate transporter 1 (VGlut1) (Nudmamud-Thanoi et al., 2008), 3) enkephalin (Kleinman et al., 1985) and neurotensin receptors (Lahti et al., 1998) and 4) fewer interneurons that express acetylcholine (Holt et al., 1999). Several studies have implicated mitochondrial abnormalities in subjects with schizophrenia (Ben-Shachar, 2002; Ben-Shachar & Laifenfeld, 2004; Kung and Roberts, 1999; Prince et al., 1999; Somerville et al., 2011a,b). These include genetic, metabolic, structural, and enzymatic alterations, many of which occur in the basal ganglia (Ben-Shachar, 2002).

Many of the postmortem pathological findings in the striatum in schizophrenia have been conducted at the ultrastructural level by Uranova and colleagues and us. Uranova and colleagues (1996, 2001, 2007) have found abnormalities in oligodendrocytes, myelin sheaths, astrocytes and synapses in the caudate nucleus and other regions. Our work has concentrated on synaptic organization, and anatomical indicators of synaptic function. Initially, we found that the size of striatal dendritic spines was smaller in schizophrenia subjects, a change that could impact synaptic efficacy (Roberts et al., 1996). Later we found more synapses in the caudate nucleus in a mixed cohort of schizophrenia patients (Roberts et al., 2005a). When examining the patch and matrix compartments, these synaptic changes were specific to the caudate matrix and putamen patches (Roberts et al., 2005b). The types of synapses that were increased in density were morphologically similar to glutamatergic inputs from cortex or thalamus or possibly serotonergic inputs.

1.4 Striatal connectivity

Knowledge of striatal circuitry has evolved over the decades from the idea of parallel segregated pathways (Alexander et al., 1986; DeLong & Wichmann, 2007) to functional connectivity (motor, limbic and associative) (Haber et al., 2000), patch/ matrix compartments (Gerfen 1984; Graybiel & Ragsdale 1978) and an integration of these circuits (Graybiel 2005; Joel & Weiner, 2000). Figure 1 illustrates a "simplified" diagram of striatal connections, the details of which are reviewed in our recent paper (Perez-Costas et al., 2010). Striatal patch and matrix compartments process different circuitry and subserve different functions, though there is evidence of cross-talk between these compartments (Bennett & Bolam 1994; Walker et al 1993). Striatal patches have connections to limbic brain regions (Cote et al. 1995; Gerfen 1984; Levesque & Parent 1998; Parent & Hazrati 1993) and abnormal circuitry therein could play a role in psychosis. This compartmentalization has been demonstrated with a variety of immunohistochemical markers (Graybiel & Ragsdale, 1978). Graybiel and Ragsdale (1978) defined these anatomically distinct compartments as striosomes (patches) and extrastriosomal matrix (matrix), though the presence of these compartments is less clear in ventral striatal areas in primate (Holt et al., 1997; Prensa et al., 1999a,b). These compartments differ from each other in several ways including the content of neurotransmitters, peptides and receptors (Graybiel & Ragsdale, 1978; Holt et al., 1997; Joel & Wiener, 2000), neuronal organization (Penny et al., 1988; Walker et al., 1993), connectivity (Gerfen 1984), developmental schedule (Graybiel & Hickey, 1982; van der Kooy and Fishell, 1987), and behavioral function (White & Hiroi, 1998). Moreover, the patches and matrix themselves are each inhomogeneous, with the patches having a belt and core (Holt et al., 1997; Prensa et al., 1999), and the matrix containing matrisomes, which are areas of focal afferents and efferents (Graybiel et al., 1991). Most medium spiny neurons have their local axon arborizations and dendritic trees located in the matrix or the striosomes, following
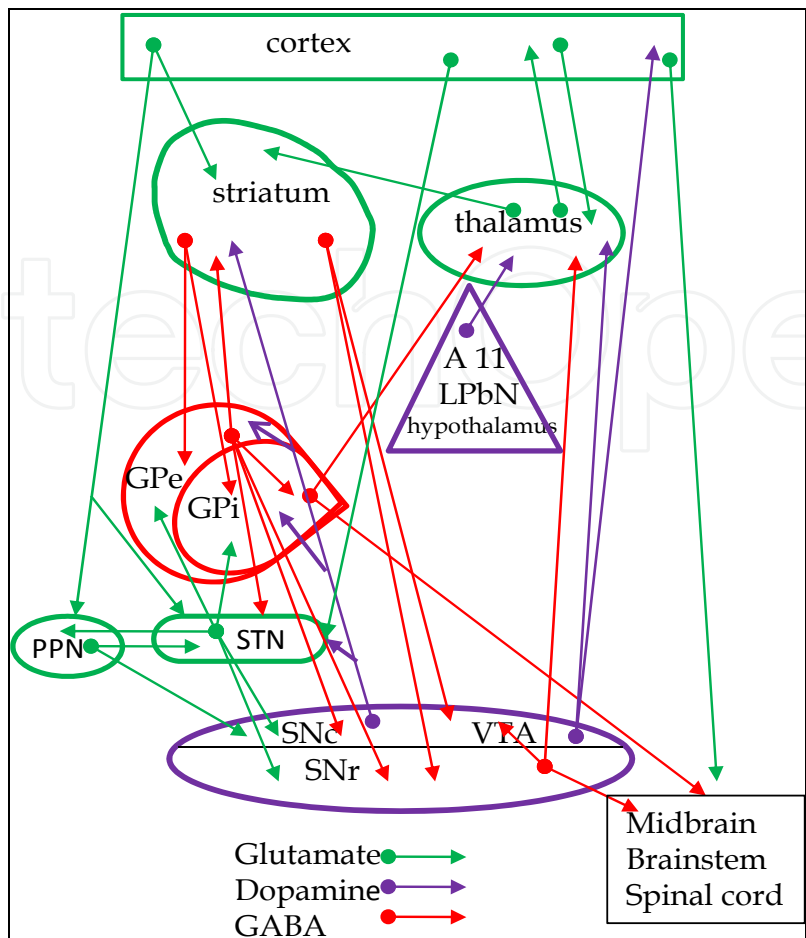
Fig. 1. Diagram of striatal connections.
Connections are shown by arrows: green for excitatory, red for inhibitory, and purple for
dopamine. Abbreviations: A11, dopamine cell group #11; LPbN, lateral parabrachial
nucleus; GPe/GPi: globus pallidus external/internal; PPN, pedunculopontine nucleus; STN,
subthalamic nucleus; SNc/r; substantia nigra pars compacta/reticulate; VTA, ventral
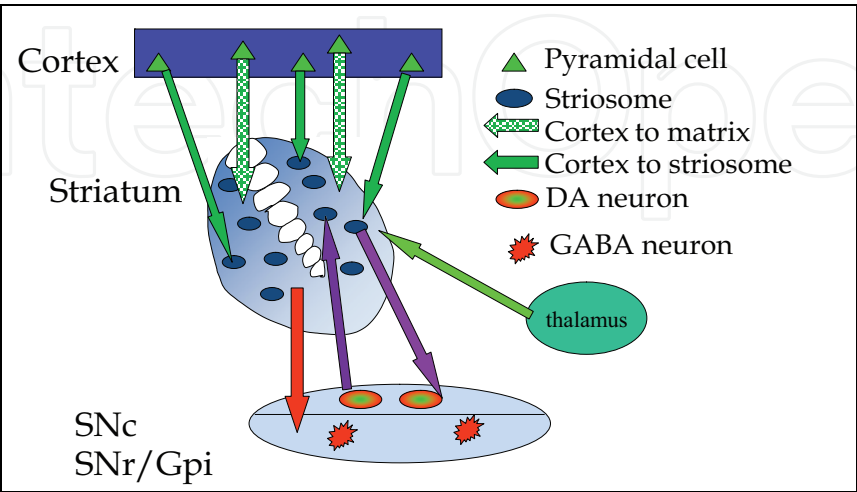tegmental area.

Fig. 2. Diagram of striosomal connections
Simplified diagram of striosomal organization. Abbreviations: same as in Figure 1.

strictly compartmental boundaries (Penny et al., 1988). However, at least in primates, there are also medium spiny neurons that do not respect these boundaries and have dendrites crossing from one compartment to the other (Walker et al., 1993; Yung et al., 1996), allowing cross-talk between compartments. Finally, ultrastructural analysis has shown that in the human striatum the matrix and striosomes have marked differences in the frequency of various types of synapses (Roberts and Knickman, 2002).

1.5 Striatal synaptic organization

In various mammalian species (Chung et al. 1977; Hassler et al. 1978; Pasik et al. 1976;) including human (Roberts and Knickman, 2002), the majority of synapses in the striatum form asymmetric synapses, characteristic of excitatory synaptic transmission. The terminals forming these synapses originate predominantly from neurons in the cortex (Kemp & Powell 1971a, b, c), with less extensive inputs arising from the thalamus (Kemp & Powell 1971a, b, c; Raju et al. 2006; Sadikot et al. 1992; Smith et al. 1994) and the raphe (LaVoie & Parent, 1990). Symmetric synapses, typical of inhibitory synaptic transmission, originate from several sources including striatal interneurons (DiFiglia & Aronin 1982; DiFiglia 1987; Ribak et al. 1979), collaterals of striatal projection neurons (Hutcherson & Roberts 2005; Pickel et al. 1980; Somogyi et al. 1981; Wilson & Groves 1980) and dopaminergic nigrostriatal neurons (Freund et al. 1984; Kubota et al. 1987a,b; Kung et al. 1998; Pickel et al. 1981). Experimental manipulations used in animal models to trace connectivity and circuits are not an option when studying human tissue. However, by examining the morphological characteristics of synapses, such as symmetry and postsynaptic target, it is possible to make educated speculations as to the origin of the neurons forming particular synapses in the human based on what is known in other species.

The main striatal targets of dopaminergic inputs are the medium spiny projection neurons (Freund et al., 1984; Kubota et al., 1987a). It has long been known that glutamatergic afferents and dopaminergic inputs converge on the same spines of these cells (Bouyer et al., 1984; Smith et al., 1994). Most thalamic inputs, except those from centromedian and parafascicular complex, also end on dendritic spines and therefore could also be modulated by dopaminergic afferents (Raju et al., 2006; Sadikot et al., 1992; Sidibe and Smith, 1999; Smith et al., 2004). This suggests that a major function of dopaminergic inputs to the striatum is the regulation of the glutamatergic pathways. Figure 3 is a schematic diagram.
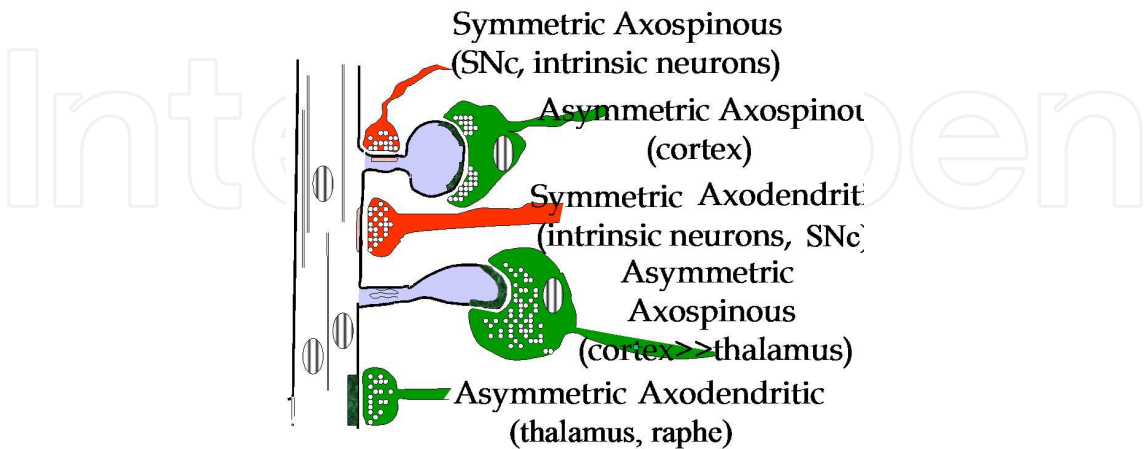

Fig. 3. Schematic illustration of synaptic connections on a medium spiny neuron.
Synapses are identified by symmetry (thickness of the postsynaptic density) and target (spine, dendrite). Green terminals are glutamatergic, while red terminals are GABAergic and also contain various peptides. The location of the neurons that form the synapses shown is indicated.

1.6 Study goals

The purpose of the present study was to compare the synaptic organization in striatal patch and matrix compartments in different subgroups of SZ, divided by treatment resistance or treatment response. We hypothesized that SZ subjects that were psychotic (off drug or poor responders) would have different alterations than treatment responsive SZ subjects. We examined striatal striosomal and synaptic organization at the electron microscopic level in postmortem striatum. These results have been presented in preliminary form (Roberts et al., 2007). We also include the results of two of our previous studies (Roberts et al., 2009; Somerville et al., 2011b) that examined treatment response/resistance and discuss the implications of all findings taken together.

2. Methods

2.1 Postmortem brain samples

Postmortem human brain tissue was obtained from the Maryland Brain Collection (MBC). The tissue was collected with family permission within 8 hours of death from subjects with schizophrenia (SZ) (n=14) and normal controls (NC) (n=8) (Table 1). The NCs had no history of central nervous system or neurological diseases and were matched to the SZ subjects for age, gender, postmortem interval and race when possible. Drug therapy, duration of illness and other medical details were obtained from hospital charts, autopsy reports and family interviews. The diagnosis of schizophrenia was made by two research psychiatrists according to the DSM-IV criteria using the Diagnostic Evaluation After Death (DEAD) (Salzman et. al., 1983) and the Scheduled Clinical Interview for the DSM III-R (SCID) (Spitzer et. al., 1992). The diagnoses of treatment response versus treatment resistance was made according to the following criteria (Conley, 2001; Conley & Kelly, 2000) which is a modification of the Kane criteria (Kane, 1988): 1) Presence of a drug-refractory condition,

	NCs (n=8)	SZ: Treatment Responders (n=8)	SZ: Resistant & Off APDs (n=6)	*df (t or F)p value*
Age in years	43±17	52±11	44±10	23 (0.775) <0.474
Race	3AA, 5C	4AA, 4C	2AA, 4C	23 (0.488)<0.620
Gender	5M, 3F	5M, 3F	2M, 4F	23 (0.655) <0.530
PMI in hours	5.4±1.6	4.62±1.41	5.50±2.43	23 (0.490)<0.619
pH (n=6/group)	7.03±0.3	6.97±0.26	6.93±0.24	18 (0.341) <0.716
DSM-IV	-----	4CUT, 3 P, 1unk	3CUT, 2P, 1unk	10 (-0.09) <0.930
APD	-----	6 typ , 2 atyp	1typ, 3atyp, 2off	12 (-4.128) <u><0.001</u>
Age of onset	-----	24.4±5.8 (n=5)	21.2±6.6 (n=4)	7 (0.762)<0.471
Length of illness	-----	26.2±14.1 (n=5)	28.8±1.2 (n=4)	7 (-0.356) <0.732

Table 1. Demographic information for subjects.
Demographic information is shown for the subjects used in the synapse data, which is new data presented in this chapter. The tyrosine hydroxylase and mitochondria data are from subsets of these cases and demographics have been previously described (Roberts et al., 2009, Somerville et al., 2010a). Abbreviations: PMI, postmortem interval; APD, antipsychotic drugs; typ, typical; atyp, atypical; A, African-American; C, Caucasian; M, male; F, female; CUT, chronic undifferentiated type; unk, unknown; Not all information was known for every subject, therefore, numbers in () indicate the number of subjects where the information was known.

which is defined as at least two prior drug treatment periods of adequate length and dose with no clinical improvement; 2) Persistence of illness, defined as at least a 5-year period with no period of good social or occupational function; and 3) Presence of persistent positive psychotic symptoms (e.g., hallucinations, delusions, suspiciousness, unusual thoughts) throughout the person's life. Cases were rated for presence or absence of these three items. If all are present, a diagnosis of treatment resistance is made. If item one is not present and one or no items are present from 2 and 3, a diagnosis of treatment responsive is made. These criteria identify subjects who did not respond to repeated trials of additional antipsychotic drugs but can respond to clozapine.

2.2 Tissue processing

Coronal blocks from the head of the caudate were dissected from fresh human brain and immersed in a cold solution of 4% paraformaldehyde and 1% glutaraldehyde in 0.1M phosphate buffer (PB), pH=7.4 for at least one week at 4°C. The striatum was washed in PB and cut on a vibratome at a thickness of 40μm into 6-12 series. One series was stained for calbindin immunoreactivity, while one series was stained for tyrosine hydroxylase immunoreactivity, as detailed below. Both series were embedded as detailed below.

2.2.1 Immunohistochemistry

To distinguish the patches from the matrix, we used calbindin-d-28K (Sigma), a calcium binding protein that preferentially stains the striatal matrix (Liu & Graybiel, 1992). Briefly, free floating sections (240 μm apart) were washed in PB (3 x 10 minutes), and incubated in 2% normal serum in PB for 30 minutes, followed by the primary antibody at a dilution of 1:20,000 for 60 hours at 4°C. Another series of sections (240μm apart) were processed from each case for the immunohistochemical localization of tyrosine hydroxylase (TH) as described previously (Kung et al., 1998; Roberts et al., 2009). Briefly, the sections were incubated in normal horse serum, followed by mouse anti-TH (Boehringer Mannheim,
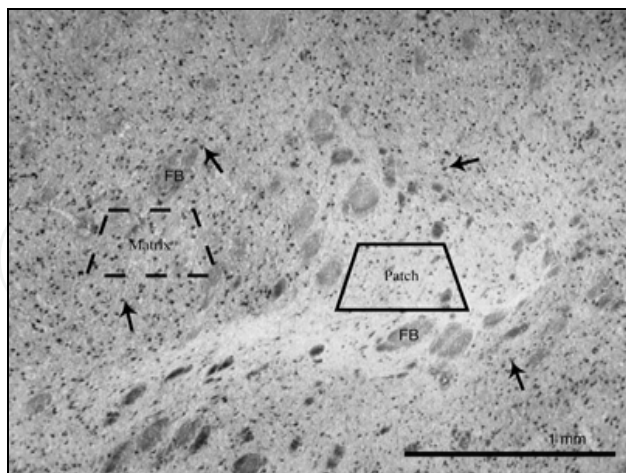

Fig. 4. Photomicrograph of calbindin immunolabeling.
Human striatum processed for calbindin immunohistochemistry to identify matrix patch compartments (defined by the darker labeling in the matrix). Note that the patch area is irregular in shape and far more lightly stained than the matrix. The trapezoids show typical areas selected for EM analysis. Arrows indicate labeled neurons. FB, fiber bundle. The scale bar = 1mm. A modification of Figure 3A in Perez-Costas et al., 2007.

Mannheim, Germany) at a dilution of 1:1,000 for 60 hours. The labeled tissue was then treated with reagents from the avidin-biotin peroxidase kit (ABC standard kit) using the recommended dilutions and times as outlined in our previous work (Roberts and Knickman, 2002; Roberts et al., 2009). Briefly, sections were then incubated in diaminobenzidine (6 mg/10 ml PB) containing 0.03% hydrogen peroxide for 5 to 10 minutes to visualize the reaction product. Controls consisted of eliminating the primary antibody but otherwise processing the tissue according to an identical protocol. Control sections did not exhibit any staining.

2.2.3 Embedding

Tissue samples were embedded using standard techniques. Briefly, the sections were rinsed in PB (3x10 minutes), immersed in 1% osmium tetroxide for 1 hour, dehydrated in ethyl alcohol, stained with uranyl acetate for 2 hours, further dehydrated in ethyl alcohol, embedded in resins on glass slides and heated at 60 °C for 72 hours. For synapse counting, at least 3 samples from different sections were randomly selected from the patches or matrix for electron microscopic analysis for each case (Figure 4). The blocks were serially thin-sectioned on an ultramicrotome at a thickness of 90nm. The average length of each ribbon was six serial sections for TH stained sections and fourteen for everything else.

2.3 Data collection and analysis

Details of the quantitative analysis of mitochondria and dopaminergic terminals have been published previously (Roberts et al., 2009; Somerville, 2011a,b). For the present analysis, in each sample, 6 photomicrographs (at a magnification of 10,000x) were taken that formed a montage. The montages were printed (final viewing magnification was approximately 25,000x), and a counting box (approximate are of $100\mu m^2$) was drawn in each. The disector stereologic technique was utilized (Geinisman et al. 1996) and described in more detail elsewhere (Perez-Costas et al., 2007). All synapses appearing in the first montage in the series and all synapses that crossed the exclusion lines in any of the series were excluded. Any profiles that appeared for the first time in subsequent montages, that met criteria, were numbered and followed in this three-dimensional reconstruction method. All synapses were quantified and then subcategorized. Thus, we identified asymmetric axospinous (AS), asymmetric axodendritic (AD), symmetric axospinous (SS), and symmetric axodendritic (SD) synapses. Then, we combined these in various ways to tally all asymmetric synapses (AS + AD), all axospinous synapses (AS + AD), all symmetric synapses (SS + SD) and all axodendritic synapses (AD + SD). The analysis of synaptic organization was performed with the experimenter blinded to the diagnosis. Synaptic data throughout the text is the number of synapses per $10\mu m^3$ ± standard deviation. Over 100 synapses or mitochondria were counted for each region per case and data are reported as the mean ± standard deviation.

2.4 Statistics

Group means and standard deviations for demographic data were obtained for each group or subgroup (Table 1). To determine whether the density of synapses was different between the controls, treatment responsive SZs and treatment nonresponsive SZs, an ANOVA followed by a posthoc t-test for multiple comparisons (least significant difference, LSD) was used. ANOVAs followed by the posthoc LSD t-test were used to determine if there were any group differences in age, PMI, race or gender between the three groups. Unpaired t-tests were used to compare parameters occurring between treatment responsive SZs and

treatment nonresponsive SZs (but not applicable to controls) such as age of onset, duration of illness, or antipsychotic drug use. Since there was a significant difference in APD use between the TR SZs and the TNR SZs, we performed a correlation analysis between APD and synapses in which we found significant measures. A Pearson bivariate correlation was used with a 2-tailed significance level of < 0.05. There were no correlations.

3. Results

Quantitative ultrastructural studies of postmortem human brain are rare outside of our laboratory, due in part to the difficulty in procuring the tissue so quickly after death. However, we have found the integrity of the tissue at the electron microscopic level to be quite acceptable for synapse identification and stereological analysis. Figure 5 shows examples of different kinds of synapses obtained from human postmortem striatum.
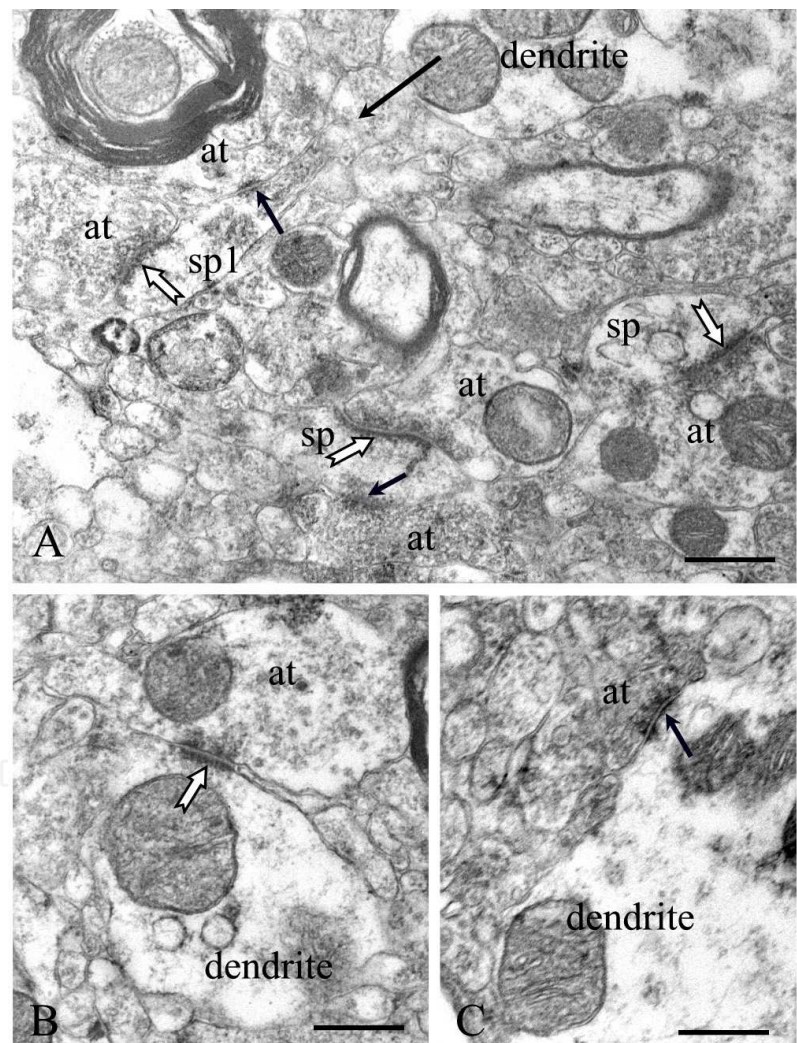

Fig. 5. Electron micrographs of postmortem human striatum.
Examples of different types of striatal synapses from control cases. Axon terminals (at) form asymmetric synapses (identified by white arrows with black borders), or symmetric synapses (black arrows). A) Several axospinous synapses are present in this field. B, C). Dendrites receive an asymmetric synapse (B) and a symmetric synapse (C). Scale bars = 0.5 μm. Figure reprinted from Figure 1 in Roberts et al., 2005b.

3.1 Striatal synaptic density

Synaptic density was determined in all three groups (controls [NCs], TR and TNR) in various regions of the striatum: caudate, putamen, patch and matrix. No matter how we divided the striatum, changes in density were found only in the asymmetric types of synapses, which signify glutamatergic inputs. In the patches (Figure 6), the data show a dichotomy in synaptic organization between TR and TNR. TNR have increased synaptic density compared to TRs or NCs for all synapses combined, asymmetric axospinous and asymmetric synapses. The density of asymmetric axodendritic synapses was increased in the TR subjects compared to both the controls and the TNR subjects. In striatal matrix (Figure 6), controls had fewer asymmetric axodendritic synapses than TR and fewer asymmetric synapses than TNR. In the matrix, controls had fewer asymmetric axodendritic synapses than TR and fewer asymmetric synapses than TNR.
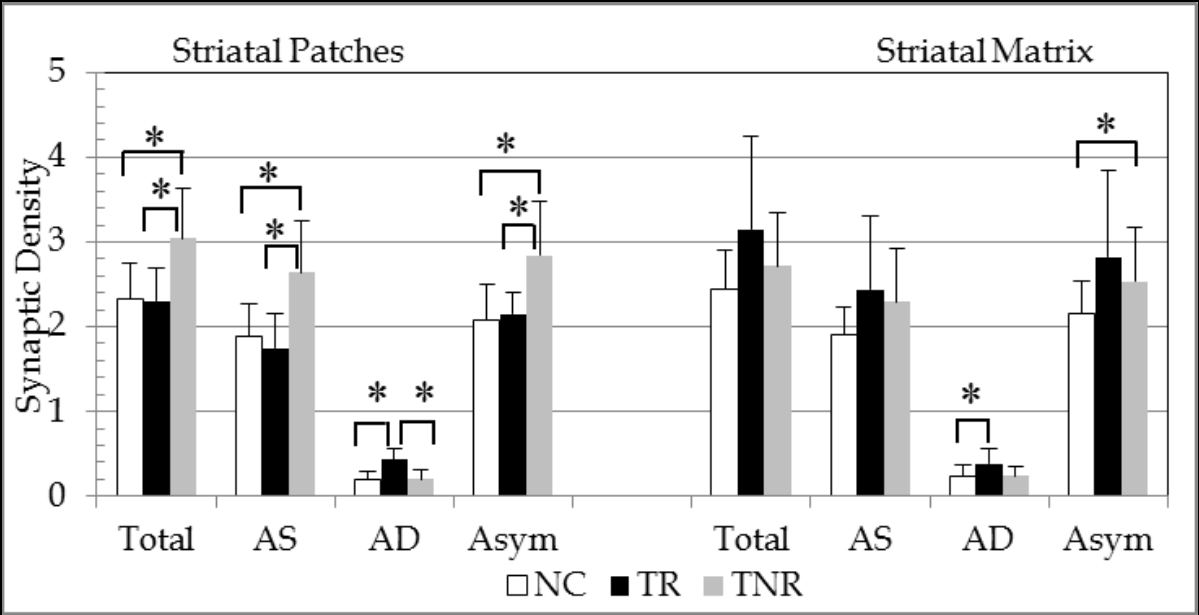

Fig. 6. Synaptic density in striatal patches and matrix.
Synaptic density (per $10\mu m^3$) is illustrated for various combinations of synapses in the patches and matrix (data combined for caudate and putamen). Total, all synapses combined, AS (asymmetric axospinous), AD (asymmetric axodendritic) and Asym (asymmetric synapses). P values are shown for LSD posthoc t-tests (*, $p<0.05$).

Synaptic density was examined in the caudate nucleus and putamen (patches and matrix combined) and differences were found here as well (Figure 7). Asymmetric axospinous synapses were higher in density in treatment non-responders significantly in the putamen, with a similar nonsignificant pattern in the caudate. The density of this type of synapse was similar between treatment responders and controls. Asymmetric axodendritic synapses were selectively elevated in the treatment responders in comparison to both controls and treatment nonresponders (Figure 7).
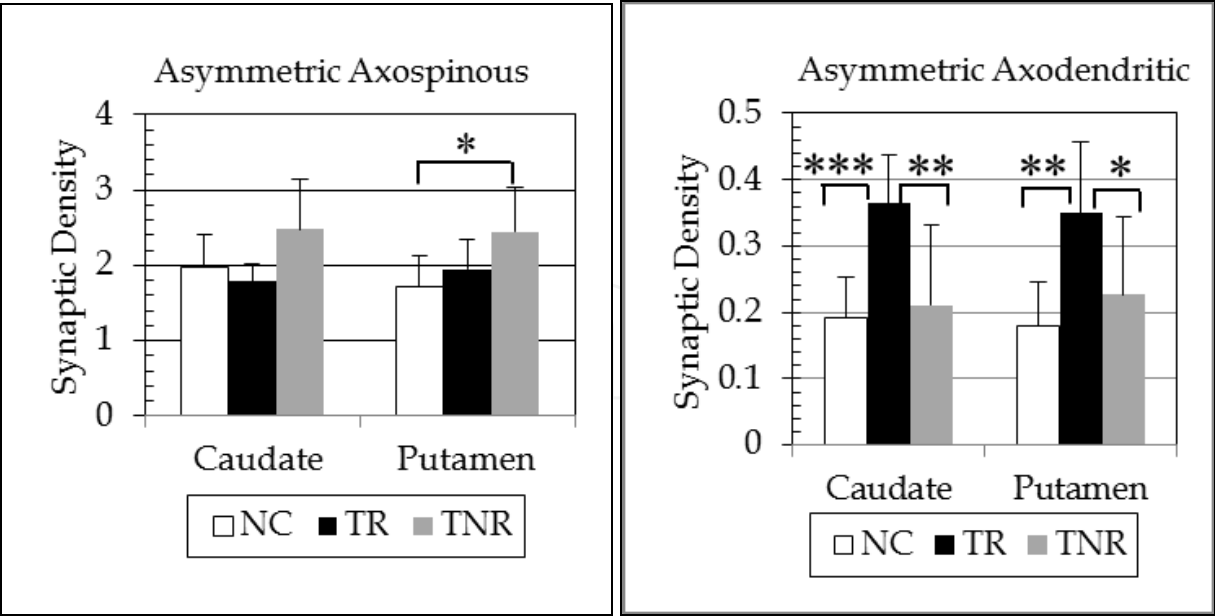
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Fig. 7. Asymmetric axospinous and axodendritic synapses in caudate and putamen.
Synaptic density is the number of synapses per 10 μm³. P values are shown for LSD posthoc
t-tests (*, p<0.05; **, p<0.008; ***, p<0.002).

3.2 Striatal mitochondria

We have previously quantified mitochondria in cohorts of schizophrenia patients as a group
and divided into various subsets (Somerville et al., 2011a,b). In a recent paper, we reported
differences in mitochondrial density in SZ subjects divided by treatment response. Here we
highlight the major changes we found in that study (Somerville et al., 2011b). The number of
mitochondria per synapse was significantly different among groups for both the caudate
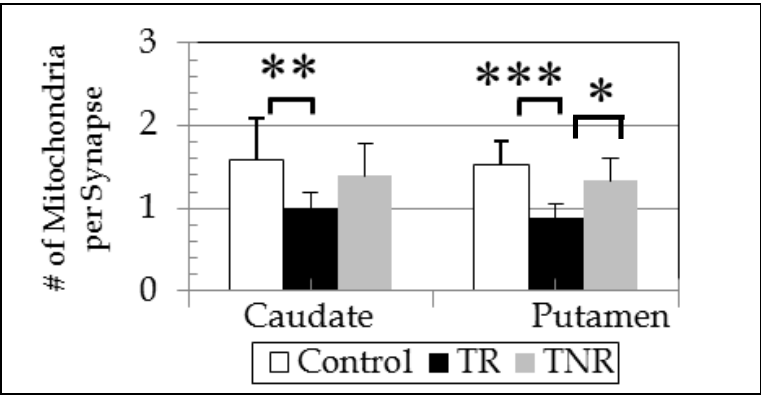

Fig. 8. Mitochondria per synapse.
Graph comparing the number of mitochondria per synapse in the caudate nucleus and
putamen. ANOVA showed significant group differences for both the caudate nucleus
(p<0.025) and the putamen (p<0.002). In the caudate nucleus, treatment responsive SZ
subjects had fewer mitochondria per synapse than that of the NCs. In the putamen, there
were significantly fewer mitochondria per synapse in treatment responsive SZ subjects
compared to both NCs and treatment resistant SZ subjects. Asterisks indicate results of LSD
post-hoc t-tests:* p< 0.05, ** p<0.01, ***, p<0.001. This graph is a modification of Figure 5
from Somerville et al., (2010b).

and putamen (Figure 8). Compared to controls, TR schizophrenia subjects had a 37-43% 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%). Our results provide further support for a biological distinction between treatment response and treatment resistance in schizophrenia.

3.3 Dopaminergic terminals in the caudate nucleus

The features of dopaminergic terminals and synapses have been described previously for normal human striatum (Kung et al., 1998) and quantified in schizophrenia (Roberts et al., 2009). Here we show the key features of those studies.
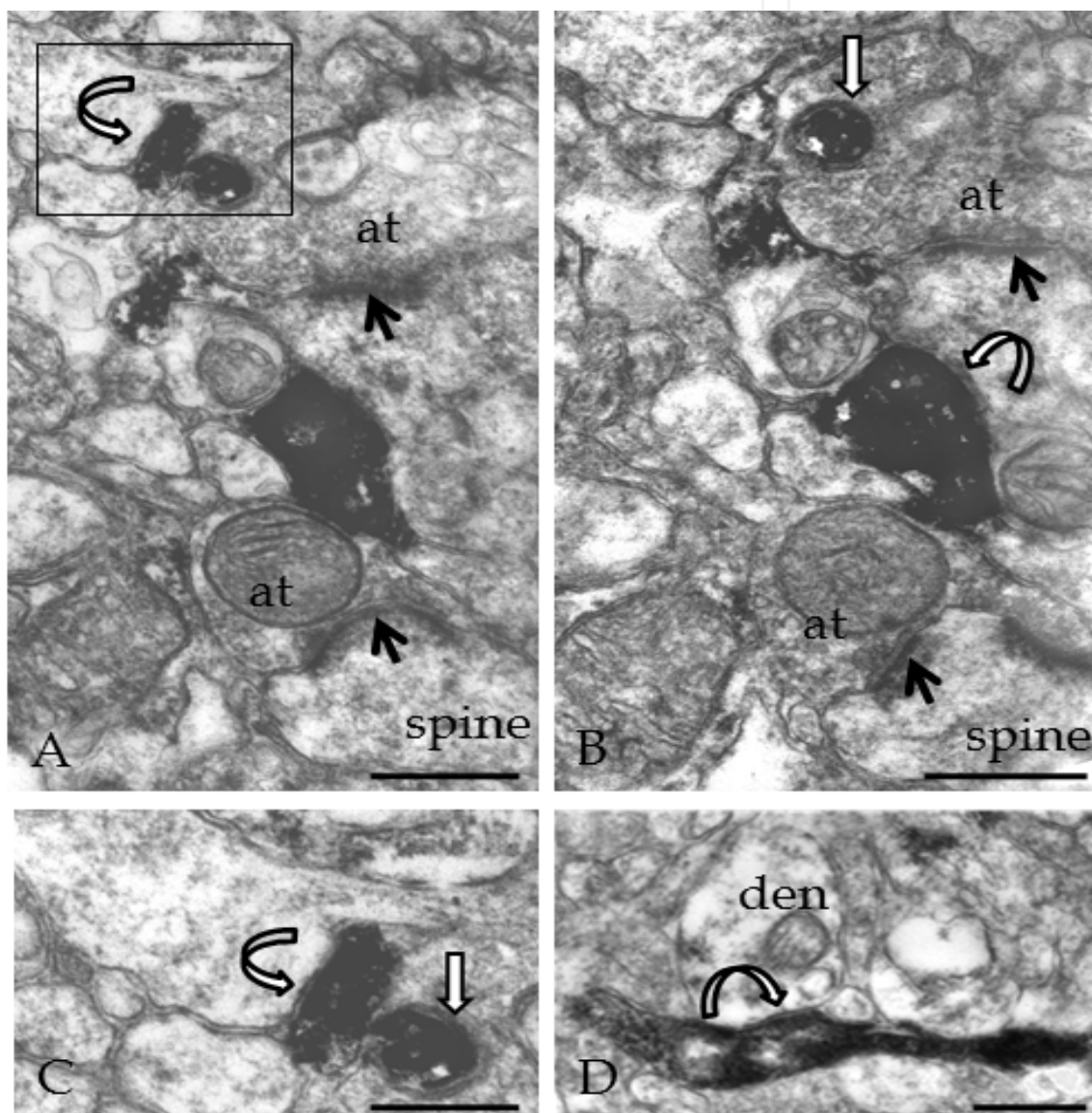

Fig. 9. Dopaminergic synapses in human caudate nucleus.
A,B) Serial sections showing several synaptic arrangements. TH-labeled axons (straight white arrows outlined in black) are adjacent to unlabeled axon terminals (at) that are forming asymmetric synapses (black arrows) with spines. TH-labeled terminals make

symmetric synapses (curved white arrows outlined in black) in both micrographs. C) Boxed area in panel A is enlarged to show the symmetric synapse (curved white arrow outlined in black). D) TH-labeled axon makes a symmetric synapse (arrow) *en passant* with a dendrite (den). A modification of Figure 1, taken from Roberts et al., 2009.

Briefly, the features of TH-labeled structures were qualitatively similar between NCs and SZ subjects. TH-labeled axons were often in close proximity to large unlabeled terminals that formed asymmetric synapses (Figure 9A,B). Synapses were formed by TH-labeled axon terminals (Figure 9A-C) and boutons *en passant* (Figure 9D). TH-labeled axon terminals formed short symmetric synapses with spines and dendritic shafts (Figure 9A-D).

Next, we quantified TH-labeled axon terminals forming synapses in schizophrenia subjects divided by treatment response or resistance (Roberts et al., 2009). The total density of TH-labeled synapses was larger in treatment responsive SZs than either the controls or the treatment resistant SZs (Figure 10). This represented a 43% and 51% larger density in the treatment responsive SZs versus the controls and the treatment non-responsive SZs, respectively. TH-labeled axodendritic synapses accounted for this difference with higher in density in treatment responsive SZs compared to treatment resistant SZ and the controls. This represented an 80% and 160% higher density in the treatment responsive SZ versus controls and treatment resistant SZs, respectively. The number of TH-labeled axospinous synapses was similar among all groups.
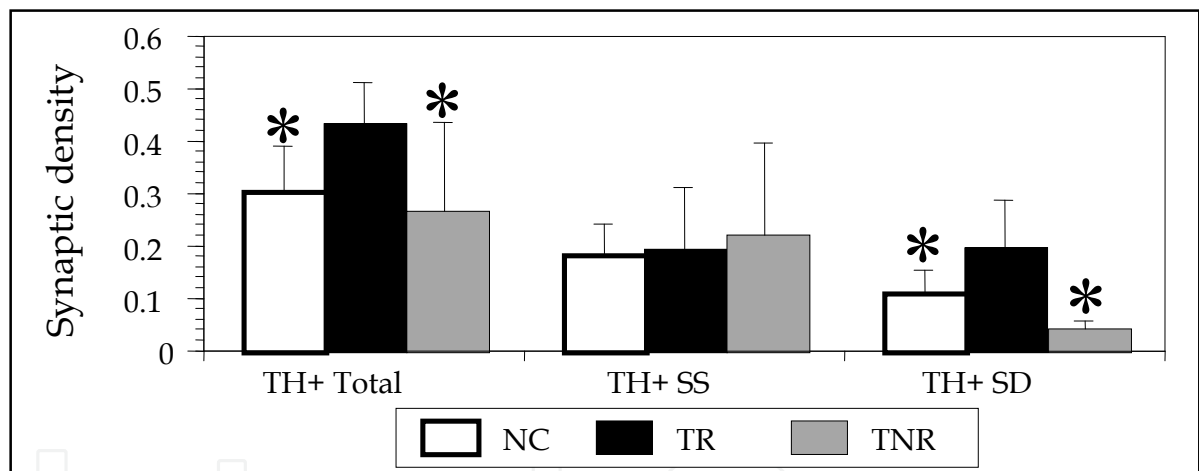

Fig. 10. Synaptic density of dopaminergic synapses in schizophrenia.
Tyrosine hydroxylase (TH) was used to identify dopaminergic synapses. Graph of the density (per $10\mu m^3$) of TH-labeled (TH+) terminals forming synapses in controls (NC), treatment responsive (TR) and treatment resistant (TNR) subjects. ANOVA results: TH+ Total, $p<0.057$; TH+ SS, $p<0.888$; TH+ SD, $p<0.017$. Total refers to all TH-labeled synapses regardless of subtype. The density of total TH+ synapses and of TH+ symmetric axodendritic (SD) synapses is greater in TR than NCs and TNR (*=$p<0.05$). A modification of Figure 2, taken from Roberts et al., 2009.

4. Discussion

This chapter presents new data on the synaptic organization in the postmortem striatum of treatment responsive vs. non responsive schizophrenia subjects, as well as presenting methods and key results of two of our previous studies on dopaminergic synapses (Roberts

et al., 2009) and mitochondria (Somerville et al., 2011b) in these same subjects. We will discuss the results of each study and then a synthesis of all the results with respect to one another and what is known in the literature.

4.1 Differential organization of asymmetric synapses in TR vs. TNR

Changes in density were found only in the asymmetric types of synapses, which signify glutamatergic inputs. In the striatal patches, which process limbic information, TNR have more cortical type synapses (AS) and more glutamatergic synapses than TR and normal controls (NC). Our findings of an increased density of synapses characteristic of corticostriatal inputs in the striatal compartment that processes limbic circuitry in TNR SZ is consistent with several reports in the literature. In vivo imaging studies have shown that regional cerebral blood flow in the anterior cingulate cortex, which is involved in limbic circuitry, is elevated in normal control people given the psychotomimetic ketamine (Lahti et al. 1995; Vollenweider et al. 1997). Similarly, a direct relationship between positive psychotic symptoms and regional cerebral blood flow has been found in the hippocampus, but only when the patients are off medication (Medoff et al. 2001). These findings suggest that psychosis is associated with more activity in the cingulate and hippocampus, an interpretation that is consistent with hyperinnervation farther downstream in the striatal patches. Moreover, psychotomimetics given to animals produce increased spine density and upregulation of markers of axon sprouting (Li et al. 2003; Ujike et al. 2002), suggesting a link between psychosis and increased numbers of axospinous synapses. Thus, we interpret that the increase in density of glutamatergic type synapses in striatal patches in SZ TNR may be related to psychosis and may be an integral part of the disease. If so, the failure to normalize this anomaly may contribute to treatment resistance and persistent psychosis.

Another change which distinguished TR from TNR was the density of asymmetric axodendritic synapses, which are typical of some cortical inputs, but mostly thalamic inputs (see Introduction for references). The TR group had more of this type of synapse than that of the controls or the TNR group. These changes were present in both patches and matrix, caudate and putamen. The increase in density may be compensatory and could play a role in treatment response.

The glutamatergic system is heavily implicated in schizophrenia and examining possible aberrant circuitry or lack of plasticity may provide new insights into treatment options (Coyle 2006; Goff & Coyle 2001; Javitt 2004; Krystal 2008). Glutamate *hypofunction* has long been implicated in psychosis and schizophrenia based on the observation that psychotomimetic agents such as PCP and ketamine block NMDA receptors; however, therapeutic manipulations to restore glutamate tone have not been successful (Javitt & Zukin 1991; Javitt 2010; Kantrowitz & Javitt 2010a, b). A glutamate *hyperfunction* hypothesis has gained recent attention. There is recent MRS evidence of increased glutamate in the striatum in drug free and treated schizophrenia subjects (de la Fuente-Sandoval et al., 2009), which validates our finding of increased glutamatergic type synapses in the striatum of subjects with schizophrenia (Roberts et al., 2005a,b). Importantly for the present results, lamotrigine, which decreases glutamate release (Yuen, 1994), augments clozapine's effects in treatment resistant SZ and attenuates ketamine induced psychosis in normal controls (Dursun & Deakin 2001; Tiihonen et al. 2003). These clinical studies linking improvement in symptoms in TNR with an agent that blocks glutamate is supportive of our data showing increased glutamate type synapses only in treatment non-responders.

4.2 Mitochondria

The main results of that study (Somerville et al., 2011b) show fewer numbers of mitochondria per synapse in treatment responsive SZs vs. NCs in both the caudate nucleus and the putamen. In addition, treatment responsive SZs had significantly fewer mitochondria per synapse than that of the treatment resistant subjects in the putamen. The observation that the treatment responders have fewer mitochondria per synapse compared to treatment resistant SZs suggests a possible compensatory mechanism that may be related to the ability to respond to treatment. Mitochondria change location in response to cellular energy demands and the stage of their own life cycle. It is unclear if the decrease in number of mitochondria per synapse is a reflection of death, fewer numbers, failure of the mitochondria to move from the cell bodies of origin or overall decreased function and return to the soma. In the same cases as those in the present paper we have shown an increase in the density of synapses in the caudate nucleus and putamen patches (Roberts et al 2005a,b). Synapses need energy to form and to function properly (Wong-Riley, 1989). The decrease in density of mitochondria in terminals forming synapses may be an adaptive response to normalize overactive neurotransmission. Future studies will address if the number of mitochondria is decreased in particular populations of synapses. Based on our data, mitochondrial density at the synapse is differentially affected in SZ according to treatment response. Understanding the role that mitochondria might play in SZ could lead to better comprehension of the mechanisms of APDs to alleviate psychotic symptoms and alter brain metabolism, and what goes awry in treatment resistance.

4.3 Dopaminergic synapses

The main results of that study (Roberts et al., 2009) showed that treatment responsive SZs have more dopaminergic synapses, as identified by TH-labeled terminals, than do treatment resistant SZs or controls. These changes were specific for the axodendritic subtype of TH-labeled synapses. Several imaging studies have demonstrated enhanced dopamine release in response to an amphetamine challenge in drug free SZ subjects (Abi-Dargham et al., 1998; Breier et al., 1997; Laruelle et al., 1996, 1999; Lindstrom et al., 1999) or neuroleptic naïve SZ subjects (Laruelle et al., 1999) compared to controls. Importantly, drug free patients who eventually responded to antipsychotic drugs had elevated dopamine release compared to those subjects who never respond to treatment (Abi-Dargham et al., 2000). The higher density of dopaminergic synapses in TR SZs may explain the results of *in vivo* studies that have measured dopamine content in live patients. However, more dopaminergic synapses may not relate to higher tonic dopamine levels. There could be several other explanations for these data, including but not limited to: differential affinity for dopamine receptors, different postsynaptic mechanisms, and/or different amounts of dopamine. Differential blockade of D_2 receptors does not appear to be responsible since treatment resistant SZs have 95% D_2 receptor occupancy (Coppens et al., 1991). The results of our study suggest that one anatomical underpinning of TR may a higher density of terminals containing dopamine.

4.4 Are ultrastructural changes related to state, trait or medication?

We have previously discussed the relationship of medication on our findings (Roberts et al., 2009; Somerville et al., 2011b). Importantly for the present data sets is the potential problem that the APDs taken by subjects in the TR and TNR groups were statistically different.

However, a regression analysis of APD type and the synaptic measures in which we found differences yielded no correlations. Moreover, APDs only help positive symptoms, and studies now show that with the exception of clozapine, typical and atypical APDs alleviate positive symptoms to the same extent (Kane et al., 2008; Lieberman et al., 2005; McEvoy, 2006; McEvoy et al., 2006). Therefore, even though the SZ subgroups were composed of different numbers of subjects on typical versus atypical APDs, the possibility that the difference in results between the groups is related to medication seems unlikely.

An important issue still unresolved in these sets of experiments is if the ultrastructural features that distinguish TR from TNR are present before or at disease onset, or reflect the ability or lack thereof to respond to treatment. Since psychotomimetics induce increased spine density and upregulation of markers of axon terminals (Li et al. 2003; Ujike et al. 2002), the increase in density of glutamatergic type synapses in SZ TNR may be related to psychosis. The interpretation we favor at this time is that the increased synaptic density in TNR reflects an integral part of the disease, which these subjects fail to normalize, and this lack of plasticity contributes to treatment resistance and persistent psychosis. With regard to our findings of increased dopaminergic synapses in TR cases, but normal numbers in TNR cases, our results are consistent with the dopaminergic hypothesis of schizophrenia and what has been shown with live people who are TR. SZ subjects who eventually respond to APDs have more striatal dopamine, while those SZ who remain TNR have normal levels. It is therefore not surprising that the TNR, who have normal levels of striatal dopamine, but who are psychotic, are not helped by drugs that block dopamine. The road to psychosis for TNR may be different from those subjects who are TR, and may include glutamate abnormalities in striatum. Of the three studies reported or reviewed herein, the mitochondria data is the hardest to understand. It remains to be determined whether the TRs, who have fewer mitochondria per synapse compared to TNR and NCs, have that feature at the onset of disease or if this is a compensatory mechanism that may be related to the ability to respond to treatment.

5. Conclusion

Our previous studies has shown that compared to controls, the striatum of SZ subjects has increased synaptic density, decreased spine size, and changes in mitochondrial distribution. In the studies summarized herein, we show differential changes in ultrastructural organization that distinguish treatment responsive from nonresponsive SZ subjects. Our postmortem results are consistent with in vivo studies suggesting a biological basis to treatment response and resistance. We hypothesized that SZ subjects that were psychotic (off drug or nonresponders) would have different alterations than treatment responsive SZ subjects. Striatal synaptic organization has been worked out in animals and by identifying morphological features of the synapse, it is possible to infer connectivity and function. In the striatal patches, which process limbic information, TNR seem to have more cortical type synapses and more glutamatergic type synapses than TR and normal controls (NC). The abnormal density of corticostriatal inputs in areas that process limbic information in TNR may be an integral part of the disease, as psychosis is linked to abnormally large amounts of synapses. If so, the failure to normalize this may contribute to treatment resistance and persistent psychosis. TR subjects have normal amounts of corticostriatal type synapses and

either have normal amounts at the disease onset or may have abnormally dense synapses like the TNR, but are able to normalize this measure. TRs have more synapses characteristic of thalamic inputs and dopaminergic synapses than NCs and TNRs. In addition the number of mitochondria per synapse is less than that of NCs and TNR. Increased dopamine synapses may be trait dependent as first episode SZ subjects who eventually respond to treatment have more dopamine as shown in in vivo imaging studies. Increased thalamic input and decreased mitochondria per synapse may be trait dependent as well, or may be compensatory and contribute to treatment response. Our results provide further support for a biological distinction between treatment response and treatment resistance in schizophrenia. Our data show an anatomical distinction between TR and TNR. Moreover, these data have important implications suggesting a biological basis to treatment response and resistance.

6. Acknowledgments

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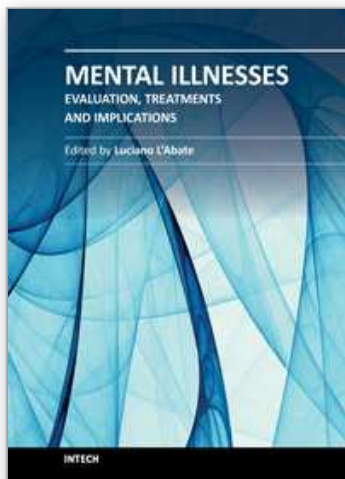
Mental Illnesses - Evaluation, Treatments and Implications

Edited by Prof. Luciano LAbate

In the book "Mental Illnesses - Evaluation, Treatments and Implications" attention is focused on background factors underlying mental illness. It is crucial that mental illness be evaluated thoroughly if we want to understand its nature, predict its long-term outcome, and treat it with specific rather than generic treatment, such as pharmacotherapy for instance. Additionally, community-wide and cognitive-behavioral approaches need to be combined to decrease the severity of symptoms of mental illness. Unfortunately, those who should profit the most by combination of treatments, often times refuse treatment or show poor adherence to treatment maintenance. Most importantly, what are the implications of the above for the mental health community? Mental illness cannot be treated with one single form of treatment. Combined individual, community, and socially-oriented treatments, including recent distance-writing technologies will hopefully allow a more integrated approach to decrease mental illness world-wide.

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