Lack of Change in Markers of Presynaptic Terminal Abundance Alongside Subtle Reductions in Markers of Presynaptic Terminal Plasticity in Prefrontal Cortex of Schizophrenia Patients

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Background: Reduced synaptic connectivity in frontal cortex may contribute to schizophrenia symptoms. While altered messenger RNA (mRNA) and protein expression of various synaptic genes have been found, discrepancies between studies mean a generalizable synaptic pathology has not been identified.

Methods: We determined if mRNAs encoding presynaptic proteins enriched in inhibitory (vesicular gamma-aminobutyric acid transporter [VGAT] and complexin 1) and/or excitatory (vesicular glutamate transporter 1 [VGluT1] and complexin 2) terminals are altered in the dorsolateral prefrontal cortex of subjects with schizophrenia ($n = 37$ patients, $n = 37$ control subjects). We also measured mRNA expression of markers associated with synaptic plasticity/neurite outgrowth (growth associated protein 43 [GAP43] and neuronal navigators [NAVs] 1 and 2) and mRNAs of other synaptic-associated proteins previously implicated in schizophrenia: dysbindin and vesicle-associated membrane protein 1 (VAMP1) mRNAs using quantitative polymerase chain reaction.

Results: No significant changes in complexin 1, VGAT, complexin 2, VGluT1, dysbindin, NAV2, or VAMP1 mRNA expression were found; however, expression of mRNAs associated with plasticity/cytoskeletal modification (GAP43 and NAV1) was reduced in schizophrenia. Although dysbindin mRNA did not differ in schizophrenia compared with control subjects, dysbindin mRNA positively correlated with GAP43 and NAV1 in schizophrenia but not in control subjects, suggesting low levels of dysbindin may be linked to reduced plasticity in the disease state. No relationships between three dysbindin genetic polymorphisms previously associated with dysbindin mRNA levels were found.

Conclusions: A reduction in the plasticity of synaptic terminals supports the hypothesis that their reduced modifiability may contribute to neuropathology and working memory deficits in schizophrenia.

Key Words: Complexin, dorsolateral prefrontal cortex, GAP43, neuronal navigator, schizophrenia, synapse

While the primary etiology of schizophrenia remains elusive, a dysregulation of synaptic connectivity of the frontal cortex may underlie the pathology and symptoms of the disease (1–4). Structural magnetic resonance imaging studies suggest a reduced frontal gray matter volume in schizophrenics (5,6) and increased packing density of cells (7,8) implicates a reduction in volume of the neuropil, where synapses are found (9). However, at the molecular level, there is a lack of consistent evidence of altered dorsolateral prefrontal cortex (DLPFC) expression of the synaptic membrane protein synaptophysin, considered one of the most valid markers of synapse density (10–12). To date, 8 of 10 studies report synaptophysin expression as unchanged in the DLPFC in patients with schizophrenia (13–20), while 3 of 10 report a reduction in synaptophysin in synaptosomal fraction or by immunohistochemistry in prefrontal cortex (18,21,22). This discrepancy suggests that synaptic reduction may not be widespread anatomically, may not be generalizable to all patients with schizophrenia, or that synaptic proteins may be reduced in a subset of terminals with a compensatory increase in others. Our previous study of five makers of presynaptic terminal proteins in patients with schizophrenia demonstrated that only vesicular associated membrane protein 1 (VAMP1) was significantly reduced (16); however, it is unknown if this fairly ubiquitous presynaptic terminal protein is reduced at the messenger RNA (mRNA) level in the DLPFC.

Considering that one of the most prominent pathologies in schizophrenia involves a deficit in gamma-aminobutyric acid (GABA)ergic interneurons or changes in factors involved in GABA neurotransmission (i.e., reduction in glutamic acid decarboxylase 67, GABA transporter 1, and GABA receptor subunits [23–29]), this may suggest that inhibitory terminals are preferentially affected in schizophrenia. Indeed, complexin 1 protein, enriched in inhibitory terminals, may be reduced in schizophrenia (30); however, mRNA is unchanged (31). Thus, the balance of inhibitory to excitatory synaptic terminals might be altered in the DLPFC in schizophrenia; however, evidence suggests that there may also be a reduction in excitatory terminals with decreased spine density (32,33) and reduced expression of vesicular glutamate transporter 1 (VGluT1) and complexin 2 (enriched in excitatory terminals) in schizophrenia (31). This leaves the relative contribution of inhibitory and excitatory terminals to cortical synaptic change in schizophrenia, as well as the nature of any synaptic loss, unresolved. In this study, we examined the expression of multiple synaptic markers in one of the largest schizophrenia cohorts studied to date to test if putative synaptic changes may preferentially involve either inhibitory terminals (indexed by vesicular GABA transporter [VGAT] and complexin 1) or excitatory terminals (VGluT1 and complexin 2) to determine which are most altered.

It is possible that overall synaptic density is unchanged, while synaptic plasticity is reduced in patients with schizophrenia, as

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Received May 13, 2010; revised Sep 14, 2010; accepted Sep 16, 2010.

0006-3223/$36.00
doi:10.1016/j.biopsych.2010.09.036

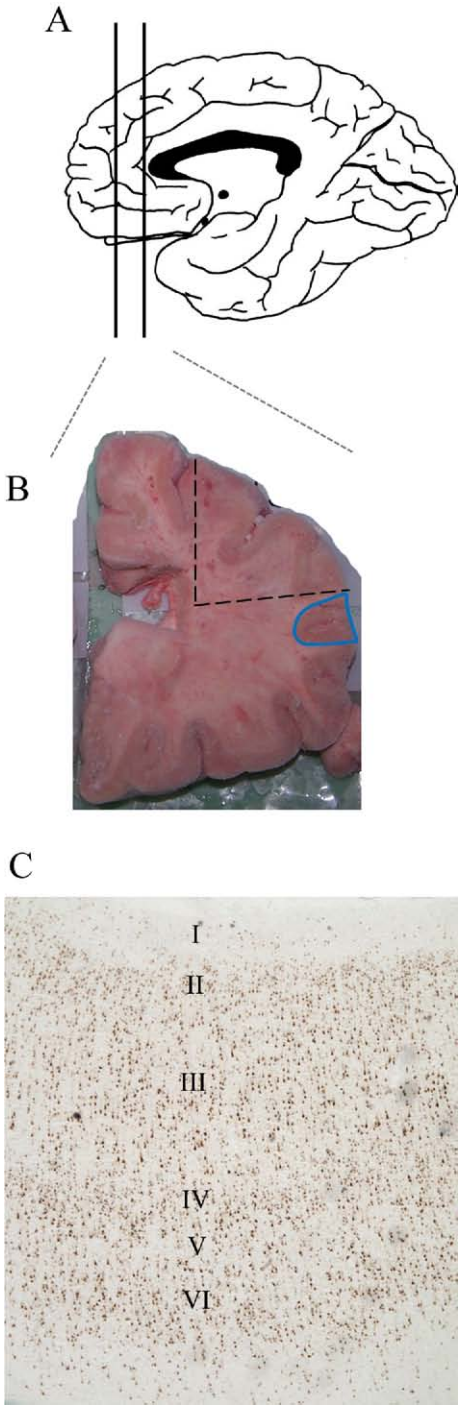

Figure 1. Isolation of dorsolateral prefrontal cortex tissue. Coronal blocks of approximately 1 cm thickness were cut from hemisected brains and frozen. The coronal block rostral to the corpus callosum or containing the genu of the corpus callosum **(A)** was used to dissect gray matter tissue containing typically inferior frontal sulcus, ventral middle frontal gyrus, and dorsal inferior gyrus (blue region in **[B]**). Fourteen micrometers frozen coronal sections were cut from adjacent tissue (dotted region in **[B]**) and Neuronal Nuclei immunohistochemistry was used to determine Brodmann area 46 cytoarchitecture **(C)**.

growth associated protein 43 (GAP43) mRNA has been reported to be reduced in the DLPFC (34) and other telencephalic areas (35–38); however, GAP43 protein has also been reported as unchanged or increased in schizophrenia (16,17,22,36,39,40). Thus, we sought to measure the expression of markers associated with synaptic plasticity/neurite outgrowth (GAP43 and neuronal navigators 1 and 2 [NAV1 and NAV2]) to determine if synaptic plasticity may be altered in our cohort (41).

It is unclear to what extent putative synaptic pathology is directly related to the etiology of schizophrenia. While multiple studies suggest that schizophrenia susceptibility genes encode proteins with synaptic function, one of the most replicated, dysbindin, is localized to the synapse (reviewed by [42]). We sought to replicate the reduction in dysbindin mRNA expression in the DLPFC (20,43) and to determine if dysbindin mRNA levels may relate to dysbindin genotypes (20,44) and/or may correlate with synaptic pathology, as has been previously found in the hippocampus (43,45). We chose this subset of mRNAs encoding presynaptic proteins based on the criteria that they were representative of either inhibitory or excitatory terminals and/or that they were previously reported to be altered in schizophrenia, with the aim to replicate findings in an additional postmortem cohort. Neuronal navigator 1 was chosen as a further candidate indicative of plasticity to corroborate or refute any GAP43 findings.

Methods and Materials

Human Postmortem Brain Tissue

Tissue from the DLPFC of schizophrenia patients and matched control subjects was obtained from the New South Wales Tissue Resource Centre (Sydney, Australia; University of New South Wales Human Research Ethics Committee #HREC07261). This cohort consisted of 30 individuals diagnosed with schizophrenia and 7 individuals diagnosed with schizoaffective disorder with tissue from 37 control subjects, where individuals were matched according to brain pH, age at death, RNA integrity number (RIN), and postmortem interval (PMI) and groups did not differ on these factors (Table S1 in Supplement 1), as described previously (41). All schizophrenia and schizoaffective cases fulfilled the criteria of the DSM-IV of the American Psychiatric Association. All subjects were prescribed antipsychotics at the time of death and the majority of patients (20 of 35 tested) had detectable levels of medication by toxicology. Control cases had no history of major psychiatric or neurological illnesses or drug abuse. Brains were hemisected and cut into coronal blocks approximately 1 cm in thickness before freezing. From the slab just rostral to or at the genu of the corpus callosum (Figure 1A), gray matter tissue was carefully trimmed from the underlying white matter with a dental drill (Cat #UP500-UG33, Brasseler, Savannah, Georgia), typically along the inferior frontal sulcus (containing ventral middle frontal gyrus and dorsal inferior gyrus [blue region in Figure 1B]). We verified that we were in a fairly consistent rostral-caudal level (from adjacent sections, dotted region in Figure 1B) by Neuronal Nuclei immunohistochemistry to confirm Brodmann area 46 cytoarchitecture was present in the block for each case (criteria adapted from [46], Figure 1C). Before RNA extraction, tissue was pulverized on dry ice. Total RNA was extracted using Trizol (Invitrogen, Carlsbad, California) from 300 mg of tissue and quality analyzed by Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, California) (41).

Immunohistochemistry

Thawed 14-μm tissue sections were fixed with 4% paraformaldehyde in phosphate-buffered saline (10 min, 4°C) and immunohistochemistry was performed using anti-Neuronal Nuclei antibody (1:1000 in diluent, Millipore MAB377; Temecula, California) as per the protocol described previously (47).