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Neuropil Pruning in Early-Course Schizophrenia: Immunological, Clinical, and Neurocognitive Correlates

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ABSTRACT

BACKGROUND: Neuropathological studies suggest neuropil reduction in schizophrenia. Altered synaptic pruning is proposed to underlie neuropil reduction. Underlying factors and clinical correlates of synaptic pruning are poorly understood. Using phosphorus magnetic resonance spectroscopy, it is feasible to assess membrane phospholipid (MPL) metabolites in the brain that specifically and sensitively reflect neuropil expansion (elevated MPL precursors) or contraction (elevated MPL catabolites).

METHODS: We examined MPL metabolites and their cognitive, clinical, and immunological correlates among 28 individuals with early-course schizophrenia (illness duration 1.99 ± 1.33 years; antipsychotic-naive, $n = 18$) and 21 controls. We acquired whole-brain multivoxel phosphorus magnetic resonance spectroscopy data from 12 unique brain regions. Interleukin-6 and C-reactive protein were assayed in the serum. Generalized linear mixed models examined case-control differences in MPL metabolites in these regions, correcting for multiple testing. Partial correlations accounting for multiple tests examined the relationship of interleukin-6 and C-reactive protein levels with MPL metabolite levels.

RESULTS: MPL catabolite levels were increased in the thalamus in schizophrenia compared with controls. Interleukin-6 and C-reactive protein levels did not show case-control differences. Interleukin-6 levels positively correlated with MPL catabolite levels in the thalamus after correcting for multiple tests. The left thalamus MPL catabolite levels correlated negatively with sustained attention (corrected $p = .039$).

CONCLUSIONS: Elevated MPL catabolite levels in the thalamus suggest increased neuropil contraction that may be related to excessive synaptic pruning. Thalamic neuropil contraction was associated with interleukin-6 levels, suggesting central pathogenic mechanisms for the inflammatory mediators. Correlation of increased thalamic MPL catabolite levels with cognitive impairments suggests clinical implications of neuropil contraction.

Keywords: Cognitive performance, Cytokines, Inflammation, Magnetic resonance spectroscopy, Neuropil, Schizophrenia

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Changes in gray matter volumes and cortical thickness and surface area are extensively reported in schizophrenia (1). Gray matter metrics represent a composite measure of axons, dendrites, synapses, glia, neuronal soma, interneurons, interneuronal space, and microvasculature (2) and hence do not reflect pathology in the neuropil, which is defined as a synaptically dense region of dendrites, unmyelinated axons, and glia with few cell bodies (3,4). Phosphorus magnetic resonance spectroscopy (^{31}P MRS) can more specifically assess neuropil by measuring membrane phospholipid (MPL) precursors (phosphocholine [PC], phosphoethanolamine, [PE]) and catabolites (glycerophosphocholine [GPC], glycerophosphoethanolamine [GPE]).

The MPLs (phosphatidylcholine, phosphatidylethanolamine) are the integral components of neuronal and glial membrane bilayers. Expansion/contraction of cell membranes that primarily occurs at the axonal endings and dendritic branches during neuropil growth/pruning are associated with elevated MPL precursors (PE, PC) or catabolites (GPC, GPE), respectively. Changes in myelination, neuronal somal size, and glial cells contribute considerably less to MPL metabolite signals on ^{31}P MRS (5). For these reasons, the major source of MPL metabolite signals is the synaptically dense neuropil (6,7). Specificity of MPL measures to neuropil pruning/formation is supported by animal lesion (5), cellular model (8), human postmortem (9), and neurodevelopmental (10,11) data. Elevated PE+PC levels are noted at the time and site of neuropil growth that increases the demand to produce MPLs (5,10,11); likewise, increased GPC and GPE levels are related to pruning (5,12). Striking changes in MPL metabolites emerged well before gray matter changes in a large cohort of healthy children/adolescents, suggesting greater sensitivity of ^{31}P MRS measures (11,13). Thus, it is feasible to more specifically conduct in vivo examination of neuropil pathology using ^{31}P MRS in schizophrenia.

Factors that contribute to neuropil pathology in schizophrenia are poorly understood. Inflammatory mediators are associated with synaptic pruning and formation (14). Mounting data support the association of inflammation with schizophrenia (15–17). Postmortem studies report activated microglia/macrophages (18,19), elevated expression of inflammatory markers in the prefrontal neurons (20) and vasculature (21), and autoantibodies against frontal (22), cingulate (23), hippocampal cortices (24), and glutamate receptors (25) in schizophrenia. Autoimmune disorders may elevate schizophrenia risk both independently (26) and in combination with infections (26). A meta-analysis noted elevated peripheral-blood inflammatory cytokine levels in schizophrenia compared with controls (27,28). Genome-wide association studies have replicated the association of the human leukocyte antigen region variants, where a large number of immune genes are located, with schizophrenia (29–31). Potential gene-environmental interactions on gray matter changes in schizophrenia are also reported (32).

Newer data in schizophrenia, including ours, associate inflammation with cognitive deficits (33,34), severity of psychopathology (35), and anatomical dysconnections (36,37). However, there are no studies on the association of inflammation with changes in neuropil in schizophrenia. Gray matter loss was associated with peripheral-blood interleukin-6 (IL-6) levels in healthy middle-aged adults (38). Enhanced progressive cortical thickness reduction in clinical high-risk subjects correlated with peripheral inflammatory mediator levels, suggesting that inflammation may increase schizophrenia risk (39).

Among the peripheral inflammatory markers, IL-6 and C-reactive protein (CRP) levels are more extensively examined in schizophrenia. IL-6 is predominantly a proinflammatory cytokine secreted by T cells and macrophages (40). CRP is a phylogenetically highly conserved acute-phase reactant and a pattern recognition molecule that is primarily expressed in liver following induction by IL-6 (41). Although extrahepatic expression of CRP in neurons (42) and lymphocytes (43) is described, plasma levels mainly reflect hepatic synthesis (41). CRP may be either pro- or anti-inflammatory, depending on the context (44). Meta-analyses report elevated IL-6 (27) and CRP (28) levels in schizophrenia, with variations related to antipsychotic use, comorbid conditions, and illness stage.

We examined MPL metabolite levels in specific brain regions across the entire brain in early-course schizophrenia compared with controls and the relationship of MPL metabolite variations with IL-6 and CRP levels; performance on sustained attention, executive function, and verbal memory tasks; and severity of psychopathology. We hypothesized that GPC+GPE levels would be elevated and/or PC+PE levels would be decreased in key regions such as the prefrontal cortex, hippocampus, striatum, and thalamus in schizophrenia compared with controls. We further hypothesized that IL-6 and CRP levels would correlate positively with GPC+GPE levels that index neuropil pruning and/or negatively with PC+PE levels that index neuropil synthesis based on the association of inflammatory mediators with smaller hippocampus (38). We predicted that the severity of psychopathology and cognitive performance would be positively correlated with MPL catabolite levels and/or negatively with MPL precursor levels.

METHODS AND MATERIALS

Clinical Evaluations

We enrolled 49 young adults (schizophrenia: $n = 28$, controls: $n = 21$) with DSM-IV schizophrenia/schizoaffective disorder between 18 and 40 years old and with an illness duration of less than 5 years from the onset of first psychotic symptom from outpatient services of the University of Pittsburgh Medical Center. The diagnosis was confirmed by administering the Structured Clinical Interview for DSM diagnosis (45) and through consensus diagnosis by experienced diagnosticians (46). Total antipsychotic dose and treatment duration were also collected. Substance abuse in the previous month or dependence 6 months before enrollment, mental retardation per DSM-IV, and serious neurological/medical illnesses were exclusion criteria.

Psychopathological and Neurocognitive Assessments

The psychopathology was rated on the Brief Psychiatric Rating Scale (47), the Scale for the Assessment of Positive Symptoms (48), and the Scale for the Assessment of Negative Symptoms (49). Sustained attention, executive functions, and verbal memory were evaluated within a week of imaging using the Go-No-Go test, the Wisconsin Card Sorting Test (WCST), and the Word List Memory Test, respectively. Accuracy and response times were used to examine sustained attention. We used perseverative error percentages on the WCST to index executive functions and trial-to-trial transfer for verbal memory and learning within the Word List Memory Test.

Imaging Procedures

Whole-brain multivoxel in vivo ^{31}P MRS data in three dimensions was collected on a 3T Siemens Tim Trio System (Siemens, Erlangen, Germany) using a dual-tuned ^{1}H-^{31}P volume head coil. The acquisition approach used the conventional chemical shift imaging (CSI) sequence, which used a single slice-selective excitation pulse to define a large axial slab and phase-encoding gradients in all three directions (termed FID_CSI on the Siemens system). Acquisition parameters were field of view = 310 × 310 × 160 mm, acquired phase-encoding steps = 14 × 14 × 8 and zero-filled to 16 × 16 × 8 (nominal voxel dimension = 1.94 × 1.94 × 2.0 cm^3), repetition time = 0.54 seconds, flip angle = 33°, reflecting the Ernst angle, where the average T1 value of phosphocreatine (PCr), PE, and PC was 3 seconds, complex data points = 2048, spectral bandwidth = 4.0 kHz, 24 averages of the CSI matrix in which the averaging was weighted to the central *k*-space points conforming to a three dimensional elliptical function and preacquisition delay time of 1.4 ms. T1-weighted magnetization-prepared rapid gradient-echo images were collected and used in the postprocessing to guide the extraction of ^{31}P MRS signal of 12 unique voxels of interest on both sides placed in anatomically specific regions, namely the dorsolateral (DLPFC) and ventral prefrontal cortex, inferior frontal cortex, ventral and dorsal hippocampus, striatum, thalamus, anterior, middle and posterior cingulate, superior temporal gyrus, and inferior parietal lobule.

The voxels of interest were predefined anatomically on a template brain and by coregistering the subject's T1-weighted