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Hypofrontality and Posterior Hyperactivity in Early Schizophrenia: Imaging and Behavior in a Preclinical Model

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ABSTRACT

BACKGROUND: Schizophrenia is a debilitating neuropsychiatric disorder typically diagnosed from late adolescence to adulthood. Subthreshold behavioral symptoms (e.g., cognitive deficits and substance abuse) often precede the clinical diagnosis of schizophrenia. However, these prodromal symptoms have not been consistently associated with structural and functional brain biomarkers, limiting the chance of early diagnosis of schizophrenia.

METHODS: Using an extensively multimodal range of magnetic resonance methods (for anatomy, metabolism, and function), we screened early biomarkers in a methylazoxymethanol acetate (MAM) rat model of schizophrenia and saline-treated control (SHAM) rats, in conjunction with immunohistochemistry, myelin staining, and a novel three-choice, reversal-learning task to identify early behavioral markers corresponding the subthreshold symptoms.

RESULTS: MAM (vs. SHAM) rats had lower/higher structural connectivity in anterior/posterior corpus callosum. The orbitofrontal cortex of MAM rats showed lower resting-state functional magnetic resonance imaging functional connectivity in conjunction with lower neuronal density, lower glucose oxidation, and attenuated neurotransmission (hypofrontality). In contrast, these measures were all higher in visual cortex of MAM rats (posterior hyperactivity), which might parallel perceptual problems in schizophrenia. In behavioral studies, MAM (vs. SHAM) rats displayed abnormal orbitofrontal cortex–mediated decision-making processes, resulting in a novel reward-sensitive hyper-flexible phenotype, which might reflect vulnerability of prodromal patients to substance abuse.

CONCLUSIONS: We identified two novel biomarkers of early schizophrenia in a preclinical rat model: hypofrontality associated with the hyperflexible phenotype, and posterior hyperactivity. Because each of these magnetic resonance methods is clinically translatable, these markers could contribute to early diagnosis and the development of novel therapies of schizophrenia.

Keywords: Decision making, fMRI, GABA, Glucose oxidation, Glutamate, Reinforcement learning

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Schizophrenia is a debilitating neuropsychiatric disorder that affects ~1% of the world's population (1). In developed countries, it is now a leading cause of disability and premature death and warrants appropriate clinical management (2). The symptomatology of schizophrenia includes positive symptoms (e.g., auditory and visual hallucinations or delusions), negative symptoms (e.g., deficits in social interaction and sensory gating), and cognitive disruptions (e.g., impaired working memory and reversal learning). Many of these abnormalities have been related to disturbances in the neuronal differentiation and migration during brain development (3). However, the early disruption of the brain is probably not the only cause of schizophrenia because etiological studies have identified a number of environmental factors experienced later in life, such as immigration and urban life, as risk factors for the illness (4,5). Collectively, schizophrenia is now considered to be a heterogeneous neurodevelopmental syndrome consisting of multiple etiologies overlaid on the abnormal brain development.

Schizophrenia is typically diagnosed during late adolescence and early adulthood, but many behavioral abnormalities precede the clinical diagnosis. These include social withdrawal, cognitive deficits, and anxiety, some of which are observed even in childhood (6–9). Also, high risk of developing schizophrenia is associated with prodromal risky behaviors including smoking (10), cannabis use (11), and alcohol dependence (12). Identifying neuroanatomical and behavioral biomarkers preceding the manifestation of the psychotic illness may assist in the early diagnosis and treatment of the disorder (13). Although several structural abnormalities, including ventriculomegaly and decreased cortical thickness, have been proposed as early biomarkers (14,15), these are not well associated with specific premorbid behavioral abnormalities.

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Adult rats prenatally exposed to methylazoxymethanol acetate (MAM), a potent genotoxin that methylates nucleic acids and alters neuronal differentiation and migration, have many behavioral impairments that are similar to individuals diagnosed with schizophrenia (16–23). Previous studies have shown that elevated subcortical activity associated with dysfunction of medial prefrontal cortex (mPFC) underlies the behavioral abnormalities of adult MAM rats compared to age-matched saline-treated control (SHAM) rats. Namely, MAM exposure on embryonic day 17 decreases the number of parvalbumin (PV)-positive gamma-aminobutyric acidergic interneurons in ventral hippocampus (vHipp), leading to the uncoordinated firing of vHipp and regions that receive projections, either directly or indirectly, from vHipp: nucleus accumbens (NAc), ventral tegmental area, amygdala, and mPFC (24–27). This process modifies the activity of dopaminergic neurons that play important roles in the altered connection strength between these regions (25,28). The aberrant activation diminishes prefrontal gamma band response during task performance, which has been proposed to underlie the cognitive impairments observed in MAM rats (26). Loss of PV-positive neurons in mPFC and orbitofrontal cortex (OFC) may also contribute to the disrupted gamma oscillations in the frontal area (29). Overall, these findings fit well with the dopaminergic hypothesis of schizophrenia, making this model attractive for preclinical applications (30–32).

Despite the progress in understanding MAM-induced dopaminergic dysfunctions, two major issues remain to be addressed in this rodent model of schizophrenia. First, brain abnormalities in the MAM model have been demonstrated predominantly in adult rats (postnatal day [PD] 90–240) (17,26,29,33–35). Only a few studies investigated young MAM animals: MAM rats in puberty and early adulthood exhibit abnormal stress sensitivity (36,37) and loss of PV-positive neurons in certain brain regions (26,38). Second, there is a lack of whole brain multimodal imaging study in MAM rats, although a diffusion tensor imaging (DTI) study previously demonstrated decreased structural connectivity in cingulum and corpus callosum (CC) in adult MAM rats (33). Surprisingly, only a single functional magnetic resonance imaging (fMRI) study has been reported for schizophrenia model rats (39). Schizophrenic symptoms are not solely attributed to dysfunctions in restricted brain regions, but arise from pervasive pathological alterations of structural and functional brain networks (40). Therefore, a whole-brain, multimodal characterization of young MAM rats may assist in the identification of early biomarkers of schizophrenia. In particular, we hypothesized that mPFC and OFC regions have impaired function in young MAM rats, resulting in the behavioral abnormality corresponding to the prodromal state of schizophrenia.

In the present study, we performed the largest ever, in vivo multimodal MR scans to characterize morphological, functional, and metabolic alterations in MAM rats in early adulthood. In vivo ^{13}C MR editing (proton-observed carbon-edited [POCE] magnetic resonance spectroscopy [MRS]) was used to assess the metabolic alterations because of its advantage in providing cell-specific tricarboxylic acid (TCA) cycle flux and glutamate-glutamine cycling rate (41,42). In conjunction with a novel three-choice, reversal-learning task, our results identified novel neuroimaging and behavioral biomarkers in the MAM model of schizophrenia that may assist in the early diagnosis and treatment of this debilitating disease.

METHODS AND MATERIALS

Animals

All procedures were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Yale University. MAM, SHAM, and naive Sprague Dawley rats were obtained from Charles River Laboratories (Wilmington, MA). See the Supplement for details.

MRI, DTI, fMRI, and MRS

MRI and DTI data were acquired at the resting state on a modified 9.4T horizontal-bore magnet with Varian spectrometer (Agilent Technologies, Santa Clara, CA) using a custom-built proton surface coil (3 by 5 cm diameter). Dexmedetomidine (0.05 mg/kg/hour) was used to sedate rats during MRI scans. The fMRI functional connectivity maps were calculated for each animal using an in-house script written in MATLAB (The MathWorks, Inc., Natick, MA). After DTI/MRI acquisition, brains were perfused and fixed in 4% paraformaldehyde for histological experiments. In vivo POCE MRS data were acquired under continuous infusion of [1,6-^{13}C]-labeled glucose through a femoral vein at 11.7T using an Agilent horizontal-bore spectrometer and a 14-mm-diameter surface coil tuned to proton frequency (499.8 MHz). Experimental details of the POCE MRS have been described earlier (43,44). See the Supplement for details of magnetic resonance scans and histology.

Behavioral Tests

Rats were either triple or quadruple housed based on sex and gestational exposure in a climate-controlled vivarium and maintained on a 12-hour light/dark cycle (lights on at 7 AM; off at 7 PM). Behavioral procedures in MAM and SHAM animals were conducted in parallel; the experiment for the naive group was conducted ~5 months after, using identical procedures. Upon arrival to the vivarium, rats were acclimated for 4 days before undergoing a mild dietary restriction to maintain a body weight of approximately 90% of their free-feeding weight for the duration of the behavioral experiments. Water was available ad libitum except during behavioral assessments (~1–2 hours/day). See the Supplement for details.

RESULTS

Brain Structural Differences in Young MAM Rats

Structural MRI revealed that MAM rats in early adulthood had smaller brain size than SHAM rats, which was apparent in the posterior region of the brain, mainly in the primary visual areas (V1) (Supplemental Figure S1A). Accordingly, MAM (vs. SHAM) rats had significantly smaller number of voxels in a localized volume located in corresponding V1 regions ($p = .01$) (Figure 1A). It was also revealed that MAM rats have larger lateral ventricles than SHAM rats in the posterior region