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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. Sub-threshold 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 multi-modal range of magnetic resonance methods (for anatomy, metabolism and function) we screened early biomarkers in 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 sub-threshold symptoms.

Results—MAM (vs. SHAM) rats had lower/higher structural connectivity in anterior/posterior corpus callosum. The orbitofrontal cortex (OFC) of MAM rats showed lower resting-state fMRI 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

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schizophrenia. In behavioral studies MAM (vs. SHAM) rats displayed abnormal OFC-mediated decision-making processes, resulting in a novel reward-sensitive hyperflexible phenotype, which might reflect vulnerability of prodromal patients to substance abuse.

Conclusion—We identified two novel biomarkers of early schizophrenia in a preclinical rat model: hypofrontality associated with the hyperflexible phenotype and posterior hyperactivity. Since 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

fMRI; glucose oxidation; glutamate; GABA; decision making; reinforcement learning

Introduction

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 since 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.

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

GABAergic 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 (VTA), amygdala and mPFC (24–27). This process modifies the activity of dopaminergic neurons which 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 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 multi-modal 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, multi-modal 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* multi-modal magnetic resonance (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 Supplement 1 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/h) 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 (MathWorks, 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 [1,6-¹³C]-labeled glucose through a femoral vein at 11.7T using an Agilent (Santa Clara, CA) 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 Supplement 1 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 h light/dark cycle (lights on at 7 am; off at 7 pm). Behavioral procedures in MAM and SHAM animals were conducted in parallel; 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 h per day). See Supplement 1 for details.

Results

Brain structural differences

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) (Figure S1A). Accordingly, MAM (vs. SHAM) rats had significantly smaller number of voxels in a localized volume located in corresponding V1 regions (Figure 1A, $p = 0.01$). It was also revealed that MAM rats have larger lateral ventricles than SHAM rats in the posterior region (Figure 1B). Ventriculomegaly has been reported for MAM rats of similar age (PD45–60) (33), whereas cortical thinning has only been observed in adult MAM rats (4–8 months) (17). Additionally we found that MAM (vs. SHAM) rats had smaller olfactory bulb (Figure S1A).

Next, the white matter integrity between MAM and SHAM rats in early adulthood was compared using DTI and luxol fast blue (LFB) myelin staining. Alterations in fractional anisotropy (FA) were regionally dependent. Compared to SHAM rats, MAM rats had higher FA in posterior CC (bregma –3.6 to –5.3 mm) containing the interhemispheric projections of the visual cortex (VC) (Figure 1C). In contrast, MAM (vs. SHAM) rats had lower FA in anterior CC (–0.2 to –1.3 mm) (Figure 1D), which contains contralateral prefrontal-striatal

projections that may be important for cognitive processes (45). Directionally encoded color (DEC) maps [similar to FA; see (46) for details] indicated that the differences in FA are mainly attributable to the altered thickness of horizontal component of the CC that contain predominantly interhemispheric connections. To evaluate these differences statistically, we quantified the number of voxels with high FA (> 0.3) in posterior and anterior CC regions where clear differences in FA were observed (Figure 1E). MAM (vs. SHAM) rats had a significantly higher number of voxels with an FA > 0.3 in posterior regions of the CC and a significantly lower number of voxels with an FA > 0.3 in the anterior regions of CC (all p 's < 0.03). LFB staining on brain sections supported the region-specific changes in CC (Figure 1F). Region-of-interest analysis and alterations in other white matter structures are described in Supplement 1.

Resting-state fMRI shows functional connectivity differences in young MAM rats

Based on the alterations in structural connectivity, we hypothesized that MAM (vs. SHAM) rats in early adulthood would have altered fMRI functional connectivity in posterior and anterior regions of the brain. To test this hypothesis, we calculated resting-state fMRI functional connectivity with seeds located in various brain regions as described below.

The most notable difference was found with a seed in the left VC: this VC seed was more highly connected with many cortical and subcortical regions in MAM compared to SHAM rats (Figure 2A, 2B). The ROI analysis followed by two-way ANOVA found significant effect of group (MAM vs. SHAM, $F_{1, 220} = 52.02$, $p < 0.0001$) and brain region ($F_{21, 220} = 9.248$, $p < 0.0001$) without an interaction ($F_{21, 220} = 0.8063$, $p = 0.71$) (Figure 2C). Significant within-region MAM/SHAM differences were detected in connections between VC and various cortical/subcortical areas (all p 's < 0.05). These results suggest that MAM rats in early adulthood may have abnormalities in VC-related behaviors.

In contrast, left OFC of MAM rats showed lower functional connectivity with other brain regions compared to SHAM rats (Figure 3A). The effects of group ($F_{1, 230} = 54.11$, $p < 0.0001$) and brain region ($F_{22, 230} = 3.137$, $p < 0.0001$) were both significant with no interaction ($F_{22, 230} = 1.150$, $p = 0.30$). Significant within-region MAM/SHAM differences were found in most cortical regions as well as midbrain, hypothalamus and dorsal hippocampus (dHipp) (all p 's < 0.05). However, with a seed in mPFC, we found no significant group effect in functional connectivity (Figure S2A). These results raise the possibility that MAM rats in early adulthood have abnormalities in OFC-mediated functions, such as behavioral flexibility and reward evaluation, rather than mPFC-mediated dysfunction. Functional connectivity between dorsal striatum and other brain regions supported this possibility (see **Results and** Figure S2B in Supplement 1).

A seed in left somatosensory cortex (SC) resulted in no significant group ($F_{1, 230} = 2.933$, $p = 0.08$) and region ($F_{22, 230} = 1.300$, $p = 0.17$) differences in functional connectivity with no interaction ($F_{22, 230} = 0.186$, $p > 0.99$) (Figure 3B). The mPFC and SC results indicate that alterations in cortical functional connectivity are not global but localized in specific regions.

When a seed was placed in the left dHipp, we found that MAM rats had significantly higher functional connectivity compared to SHAM rats (Figure 3C; $F_{1, 230} = 70.93$, $p < 0.0001$).

The effect of brain region ($F_{22, 230} = 0.9930$, $p = 0.47$) and the interaction ($F_{22, 230} = 0.2476$, $p > 0.99$) were not significant. Group differences in functional connectivity with the dHipp seed were less clear with larger individual variations compared to those with VC and OFC seeds; post-hoc analysis detected significant within-region differences only in dorsal striatum and NAc ($p = 0.04$ and 0.03 , respectively). With a left vHipp seed, MAM rats also had higher functional connectivity with other brain regions compared to SHAM rats (Figure S2C).

We also calculated functional connectivity density (FCD) maps to evaluate overall changes in functional connectivity. The FCD value of a given voxel represents the total number of functional connections to other voxels above a certain threshold (47). MAM rats had higher number of global and long-range connections compared to SHAM rats (Figure 4A, 4B). In contrast, the number of short-range connections was decreased in MAM rats (Figure 4C). These changes are consistent with brain network alterations in individuals with schizophrenia (40) and suggest that there is a reduced communication within local regions of the brain. ROI analysis of FCD maps did not find significant within-region difference possibly due to large individual variations (Figure S2D). Subcortical signals might be reduced by the use of surface coil in our fMRI analysis, but this does not greatly affect the overall conclusion.

¹³C MR editing confirms hypofrontality and posterior hyperactivity in young MAM rats

Based on our region-specific results indicating hypofrontality and posterior hyperactivity in MAM rats in early adulthood (Figure 2–4), we hypothesized that the observed changes might be due to metabolic differences across brain regions. To test this hypothesis, we measured resting-state neuronal TCA cycle flux ($V_{TCA,N}$) and glutamate-glutamine cycling rate ($V_{cyc(tot)}$) of VC, OFC, SC and dHipp in MAM and SHAM rats by *in vivo* POCE MRS. After infusion of [1,6-¹³C]-labeled glucose, clear peaks of Glu-C4 (2.34 ppm) overlapping with smaller peaks of Gln-C4 (2.43 ppm), were observed in difference spectra (Figure S3).

In line with our hypothesis, metabolic differences between MAM and SHAM animals were consistent with the resting-state fMRI functional connectivity differences. MAM (vs. SHAM) rats had significantly higher and lower $V_{TCA,N}$ and $V_{cyc(tot)}$ in the VC and OFC, respectively (Figure 5; $p = 0.04$ for both). In SC and dHipp, the metabolic fluxes did not significantly differ between MAM and SHAM rats ($p = 0.77$ and 0.74 , respectively). We also confirmed the well-known 1:1 relationship in plots of cerebral metabolic rate of neuronal glucose oxidation ($CMR_{glc(OX,N)}$) and glutamate-glutamine cycling rate (Figure S4). Immunohistochemical staining showed that MAM rats had higher and lower neuronal density in VC and OFC, respectively, which possibly accounts for the metabolic alterations (Figure S5).

Behavior reveals hyperflexibility with increased sensitivity to reward in young MAM rats

Based on the morphological, functional and metabolic results, we hypothesized that OFC-mediated behaviors would be disrupted in MAM rats in early adulthood. To test this hypothesis, the ability of MAM and SHAM rats to acquire and reverse three-choice, spatial discrimination problems using a probabilistic schedule of reinforcement was assessed

between PD 35–83. Behavior was also assessed in a group of age-matched naive rats ($n = 16$) in an independent experiment to verify that behavior of SHAM rats was intact.

Rats were trained to make sustained nosepoke responses into illuminated noseports to earn probabilistically delivered rewards (sucrose pellets). Details of the training procedure are described in the Supplement 1. Once nosepoke responding was established, rats were trained to acquire and reverse three-choice spatial discrimination problems in single 240 trial sessions (Figure 6A, 6B). The percentage of trials, in which rats chose the noseport associated with the highest probability of reinforcement, was compared across the acquisition and reversal phase between the three experimental groups (Figure 6C). There was a significant effect of phase (acquisition vs. reversal; $F_{1,282} = 323.2$, $p < 0.001$), group (MAM, SHAM and naive; $F_{2,282} = 5.70$, $P = 0.004$) and a phase-by-group interaction ($F_{2,232} = 4.43$, $p = 0.01$). Post-hoc analyses indicated that performance in the acquisition phase did not differ between groups (all p 's > 0.59). During the reversal phase, however, MAM rats chose the highest reinforced option significantly more than SHAM ($p = 0.02$) and naive rats ($p = 0.001$). Furthermore, MAM rats made significantly fewer perseverative responses in the reversal phase than SHAM ($p = 0.009$) or naive ($p < 0.001$) rats (Figure 6D), indicating that MAM rats were better at adapting their responding when the reinforcement probabilities changed (i.e., hyperflexible phenotype). There was no significant group differences in the probability of choosing the noseport associated with the intermediate level of reinforcement (all p 's > 0.73). No significant differences were found between SHAM and naive rats for any of these measures (all p 's > 0.99).

Next, we sought to characterize the behavioral processes contributing to the hyperflexibility observed in the MAM rats in early adulthood (Figure 6E). Trial-by-trial choices made during the reversal phase of the task were modeled using a reinforcement learning algorithm (48, 49) to obtain an estimate of the learning rate (α), the strength of reinforcement by reward (β_1) and the strength of the aversion from lack of reward (β_2) for each rat for each session completed. We found a significant effect of group ($F_{2,270} = 17.81$, $p < 0.001$), parameter estimate ($F_{2,540} = 105$, $p < 0.001$) with an interaction ($F_{4,540} = 6.27$, $p < 0.001$). Post-hoc analyses revealed that the β_1 parameter was significantly higher in MAM rats compared to SHAM and naive rats (all p 's < 0.001). Additionally, the β_2 parameter was greater in MAM rats compared to naive rats, but not compared to SHAM rats ($p = 0.71$). No significant differences were detected between SHAM and naive rats for any of these measures (all p 's > 0.13).

Discussion

The large-scale multi-modal imaging combined with behavioral analyses identified multiple early morphological, functional, metabolic and behavioral alterations in MAM rats that consistently indicated hypofrontality (i.e., OFC showed lower structural/functional connectivity, neuronal density, glucose metabolism, glutamate-glutamine cycling rate associated with OFC-mediated decision-making process) and posterior hyperactivity (i.e., the same measures were all higher in VC). These early markers could advance our understanding the neurodevelopmental processes that underlie schizophrenia, and, at the same time, could also contribute to early diagnosis and the development of novel therapies.

Multi-modal imaging methods used in this study have the potential to detect even earlier functional and metabolic markers of schizophrenia than those identified in this study. For example, since the formation of CC is complete by PD15 in rodents (50), the interhemispheric changes in functional connectivity of MAM rats might also appear by this age. In addition to proposed early structural markers (14, 15), functional and metabolic changes would help characterize markers of early schizophrenia.

In reward-based decision-making tasks, dopaminergic input into dorsal striatum and NAc play a role in reinforcement processing (51). The OFC is hypothesized to integrate multiple reward-related information from sensory and reward systems, which is subsequently used by the PFC in reward valuation and adaptive decision-making (52). Therefore, OFC hypometabolism and decreased striatal-OFC functional connectivity are likely related to the behavioral abnormalities observed in MAM rats. The increased sensitivity to reward has also been observed in the prodromal stage of schizophrenia and is hypothesized to be a potential risk factor for the development of substance dependence problems during adolescence (53, 54). Furthermore, individuals with substance abuse disorder show OFC hypometabolism and striatal dopaminergic dysregulation (55, 56). In schizophrenia, however, the relationship between OFC, striatum and reward sensitivity is less clear; the above explanation can be invoked only for a subgroup of patients with OFC hypometabolism (57, 58). Increased OFC metabolism has also been observed in schizophrenia, possibly reflecting the heterogeneous nature of this disease (59, 60).

On the other hand, the hyperflexible phenotype of young MAM rats (PD34–83) is in contrast to evidence for impaired reversal learning that has been observed in older MAM rats (PD80–120 or older) (21, 29) and in individuals with schizophrenia (61). The age-dependent decline in reward sensitivity might explain this discrepancy. Specifically, reward-seeking behaviors peak in adolescence and decline thereafter with a concomitant increase in prefrontal control and decrease in midbrain dopamine concentration (62–65). Given that increased reward sensitivity could enhance reversal learning by promoting a link between the new chosen stimulus and reward, one would expect that a hyperflexible phenotype is prominent in young MAM animals – that may be altered as the disease progresses. Indeed, two studies have reported enhanced behavioral flexibility in models of schizophrenia-like behaviors (66, 67). Longitudinal studies examining behavioral flexibility at different developmental time points in MAM-exposed rats might provide insight into the pathological progression of schizophrenia.

Another major brain abnormality observed in MAM rats in early adulthood is the VC hyperactivity, which might be related to visual perceptual problems in schizophrenia (68). Alternatively, MAM rats may correspond to a relatively minor subgroup of schizophrenic patients with visual hallucinations given reports of increased structural and functional connectivity between VC and hippocampus/amygdala (69, 70) and activation of visual areas detected by PET (71) and fMRI (72) during visual hallucination. Visual abnormalities in animal models are difficult to confirm, though positive symptoms (i.e., hallucinations) have been linked to excessive locomotor activity and such phenotypes have been argued to be associated with mesolimbic dopamine dysregulation (73). Imaging-based detection of such sensory-related symptoms would provide a new insight into animal behavioral studies.

Our multi-modal imaging of MAM vs. SHAM animals in early adulthood did not reveal clear differences for the vHipp and mPFC despite previous evidence of hyperactivity in these regions in adult MAM rats and schizophrenic patients (24–26, 29, 34). The results of the current study suggest that alterations in vHipp and mPFC are minor in the MAM rats in early adulthood since we did not observe any differences in the resting-state fMRI functional connectivity between the experimental groups. Indeed, vHipp exhibits delayed loss of PV-positive neurons compared to dHipp (38).

A limitation of our study is the use of dexmedetomidine, an alpha-2-adrenoreceptor agonist, to sedate rats so that spontaneous body motion and irregular breathing artifacts during MRI scans are reduced. Artifacts arising from motion, breathing and heart rhythms as well as physiological changes of stress-related parameters in the awake state can have detrimental effects on functional connectivity measurements. In this regard, dexmedetomidine sedation was suitable since it retains cortical and subcortical topology of functional connectivity networks (74). Since MAM and SHAM rats were both exposed to the same anesthetic, we believe the comparative analysis reveals model-specific changes. This assertion is supported by the complementary results between anesthetized metabolic/functional scans and awake behavioral studies. While it is unlikely that alpha-2-adrenoreceptor agonist will act differently in MAM vs. SHAM rats, future MRS studies could assess these effects. Another limitation is the lack of correlative analysis of early schizophrenic markers within rats. We could not measure multiple parameters in a single rat due to lengthy experimental durations, a factor that could affect the imaging quality. Although several parameters were acquired from same cohort of subjects, the relatively small sample size allowed us to only detect group differences. Studies with larger group sizes could explore the relationship between structural, functional and metabolic alterations in MAM rats identified in the current study. Furthermore we may even consider another novel DTI parameter called permeability-diffusivity-index (PDI) (75) in future studies (see Supplement 1). While both neuronal density (Figure S5) and volume loss (Figures 1A and S1A) in MAM rats were determined in this study with immunostaining and MRI data respectively, a constraint of this study was that we could not assess innervation patterns of neurons in specific regions by microscopy. Future studies are planned to relate high-resolution PDI with neuronal innervation patterns guided in the same tissue samples.

In conclusion, we identified imaging and behavioral biomarkers of MAM rats in early adulthood. The hypofrontality observed in early adulthood may continue into adulthood given that the hypofrontality is common to adults with schizophrenia. In addition, posterior hyperactivity in young MAM rats will be a useful indicator of perceptual problems in animal schizophrenic research with adequate behavioral validation. We further propose that the hyperflexible phenotype associated with OFC hypoactivity represents a potential behavioral marker for the emergence of schizophrenia. Because all of the magnetic resonance methods used in the current study have high translational validity, multi-modal imaging will be a useful tool to investigate heterogeneous alterations in symptoms of schizophrenia, specifically at the onset of symptoms, which in turn could contribute to early diagnosis of schizophrenia leading to development of novel therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Brain morphology and structural connectivity of methylazoxymethanol-treated (MAM) rats compared to saline-treated (SHAM) rats ($n = 6$ for each group; PD50–67). **(A)** MAM rats had thinner primary visual cortex (V1) than SHAM rats. A corresponding V1 region (bregma -6.8 mm) was selected on fractional anisotropy (FA) maps computed from the diffusion tensor imaging (DTI) data. Cortical volumes, as represented by number of voxels above forceps major in the selected region, were compared between MAM and SHAM rats. MAM V1 was $\sim 7.3\%$ thinner than SHAM V1, $*P < 0.05$ (Welch's t -test, error bars represent standard errors). FA scale is unitless. **(B)** MAM rats had enlarged lateral ventricles (LV, arrowheads). Representative MAM minus SHAM structural MRI images masked at $P < 0.05$ are overlaid on average SHAM anatomical images. Because ventricles have higher intensity than tissue regions, high values represent ventricle enlargement. Ventricle size was quantified as number of high intensity (> 2250) voxels within the ventricle region of the Paxinos and Watson atlas for three regions (bregma 2.2 to -0.1 mm, -0.1 to -2.4 mm, and -2.4 to -4.5 mm). $*P < 0.05$, Welch's t -test. **(C, D)** Average FA and DEC maps of MAM and SHAM rats. Arrowheads indicate corpus callosum (CC), where marked differences in FA were observed. **(E)** Number of voxels with FA > 0.3 in posterior CC (bregma -3.6 to -5.3 mm) and anterior CC (bregma -0.2 to -1.3 mm) were compared between MAM and SHAM rats. Error bars represent standard errors. At a threshold FA value of 0.3 , MAM (vs. SHAM) rats had higher number of voxels in posterior CC ($***P < 0.01$) and MAM (vs. SHAM) rats had lower number of voxels in anterior CC ($*P < 0.05$) (Welch's t -test). **(F)** Luxol fast blue (LFB) myelin staining compared with FA maps of posterior CC and anterior CC. Red and blue bars indicate the thickness of CC of MAM and SHAM rats, respectively. These bars are also shown on the right margin for visual comparison. Scale bar (black), 1 mm. See Figure S1 for other morphological changes.

Figure 2.

Comparison of fMRI-measured resting-state functional connectivity with a seed in the left primary (V1) and secondary (V2) visual cortex for MAM ($n = 7$) and SHAM ($n = 5$) rats (PD50–67). (A, B) Average z-maps of MAM and SHAM rats, where the seed region is represented by red. (C) Analysis of different region of interest (ROI) of z-maps using a high-resolution atlas. Error bars represent standard errors. Two-way analysis of variance (ANOVA) followed by Welch's t-test shows that MAM rats had higher functional connectivity with V1/V2 seed ($*P < 0.05$). The gray matter ROIs include: primary visual cortex (V1), secondary visual cortex (V2), somatosensory cortex barrel fields (S1 BF), primary somatosensory cortex (S1), secondary somatosensory cortex (S2), primary motor cortex (M1), secondary motor cortex (M2), S1 fore-limb (S1 FL), S1 hind-limb (S1 HL), prelimbic cortex (PrL), infralimbic cortex (IL), cingulate cortex (Cg cortex), orbitofrontal cortex (OFC), midbrain, substantia nigra (SN), septum, hypothalamus, striatum, diencephalon, dorsal hippocampus (dHipp), ventral hippocampus (vHipp), pallidum, accumbens, amygdala and bed nucleus of the stria terminalis (BNST).

Figure 3. Comparison of fMRI-measured seed-based resting-state functional connectivity between MAM and SHAM rats. Seeds are located in orbitofrontal cortex (**A**), somatosensory cortex (**B**), and dorsal hippocampus (**C**) for MAM (n = 7) and SHAM (n = 5) rats (PD50–67). See Figure S2 for comparison with other fMRI seeds. Error bars represent standard errors. Two-way ANOVA followed by Welch’s t-test shows that MAM rats had lower and higher functional connectivity with OFC and dHipp seeds, respectively ($*P < 0.05$). MAM and SHAM rats had comparable functional connectivity with somatosensory cortex seed. See Figure 2 legend for abbreviations.

Figure 4.

Comparison of fMRI-measured resting-state functional connectivity density (FCD) for MAM (n = 7) and SHAM (n = 5) rats (PD50–67). FCD was calculated with correlation threshold of 0.6. Global (A), long-range (B), and short-range (C) FCDs were calculated by considering connections longer than 2 mm to be long-range. Average FCD maps are shown with histograms for number of voxels. MAM rats had higher FCD values in the global and long-range, but not in the short-range.

Figure 5.

Comparison of metabolic fluxes for MAM and SHAM rats. Neuronal tricarboxylic acid (TCA) cycle ($V_{TCA,N}$) (**A**) and total glutamate-glutamine cycling ($V_{cyc(tot)}$) (**B**) at the resting-state were measured by *in vivo* proton-observed carbon-edited (POCE) MRS. The metabolic fluxes, $V_{TCA,N}$ and $V_{cyc(tot)}$, were measured in visual and somatosensory cortices ($n = 6$ for MAM; $n = 5$ for SHAM; PD50–78), OFC ($n = 5$ for MAM; $n = 5$ for SHAM; PD68–83) and dHipp ($n = 4$ for MAM; $n = 5$ for SHAM; PD68–83). See Figure S3 for details of POCE MRS data. Error bars represent standard errors. Student's t-test showed that MAM rats had higher and lower metabolic values (i.e., $V_{TCA,N}$ and $V_{cyc(tot)}$) in visual and orbitofrontal cortices, respectively (* $P < 0.05$). MAM and SHAM rats had comparable metabolic values (i.e., $V_{TCA,N}$ and $V_{cyc(tot)}$) in dHipp and somatosensory cortex. Coupling metabolic fluxes, $V_{TCA,N}$ and $V_{cyc(tot)}$, across regions is shown in Figure S4.

Figure 6.

Performance of MAM ($n = 10$), SHAM ($n = 11$) and naive ($n = 16$) rats (PD34–83) in the probabilistic reversal-learning task. **(A)** Schematic representation of the probabilistic reversal-learning task (PRL). See Methods and Materials in Supplement 1 for details. **(B)** The schedule of reinforcement used in PRL task. The first 120 trials of these sessions were the “Acquisition Phase” during which rats had to learn which one of the three noseport apertures was associated with the highest probability of reinforcement (72% versus 8% and 24%). Assignment of reinforcement probabilities was randomly assigned each session by the program. Once rats had completed 120 trials, the reinforcement probabilities were reversed between two of the noseport apertures: the noseport associated with the highest probability of reinforcement during the Acquisition Phase (72%) was now associated with the lowest probability of reinforcement (16%) and the noseport associated with the lowest probability of reinforcement during the Acquisition Phase (8%) was now associated with highest probability of reinforcement (64%). The noseport associated with an intermediate probability of reinforcement during the Acquisition Phase (24%) remained at an intermediate level of reinforcement during the Reversal Phase (36%). Choice behavior of rats was assessed for an additional 120 trials or until 76 min had lapsed, whichever occurred first. Rats completed between 10–14 of these sessions prior to undergoing the MR procedures. **(C)** The probability of choosing the highest reinforced option during the reversal, but not acquisition, phase was significantly greater in MAM rats compared to SHAM and naive rats. **(D)** The probability of making a perseverative, but not an

intermediate response, during the reversal phase was lower in MAM rats than in SHAM and naive rats. (E) Computational analyses of choices made during the reversal phase indicated that while the learning rate (α) was the same in each group, the β_1 (e.g., reinforcing strength of reward outcome) parameter was greater in MAM rats compared to SHAM and naive rats and that the β_2 (e.g., aversive strength of a non-reward outcome) parameter was greater in MAM rats compared to naive, but not SHAM, rats. Two-way ANOVA followed by Sidak's multiple comparison test shows the statistical differences. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Bars represent standard errors. See Figure S5 for details on other comparisons of behavioral results between MAM and SHAM rats.